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GENETIC DIVERSITY OF *Alternaria solani* AND *Phytophthora infestans* ISOLATES AND THEIR MANAGEMENT USING CRUDE EXTRACTS AND ESSENTIAL OILS OF SELECTED PLANTS IN KENYA

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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award.

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DEDICATION

This work is dedicated to God my creator for giving me the strength and to my family who has been a strong pillar during the study period.

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ABBREVIATIONS AND ACRONYMS

ANOVA	-	Analysis of variance
AVRDC	-	Asian Vegetable Research Development
BP	-	Base pairs
DNA	-	Deoxyribonucleic Acid
EU	-	European Union
FAO	-	Food and Agricultural Organization
GC-MS	-	Gas chromatography – mass spectrometer
GoK	-	Government of Kenya
HCDA	-	Horticultural Crop Development Authority (now Horticultural Crops Directorate)
ITS	-	Internal Transcribed Spacer
MRLs	-	Maximum Residue Levels
MSD	-	Mass Selective Detector
PDA	-	Potato Dextrose Agar
SNK	-	Students Newman Keuls
USDA	-	United States Department of Agriculture

ABSTRACT

Kenya is rated 6th in Africa with an annual overall production of 397,007 tons of tomato fruit. Tomato production in Kenya is constrained by many biotic and abiotic factors and among them, diseases such as early and late blight caused by *Alternaria solani* and *Phytophthora infestans,* respectively. Farmers use synthetic chemicals to manage the diseases. However, there has been major concern because they are costly, toxic to human beings and the environment. Some of the products have been rejected in the international markets because of high chemical residue levels. Some of the pesticides have not been effective because of the pathogens undergoing genetic mutations that result to resistant strains. This study sought to assess the genetic diversity of pathogens causing early and late blight in tomato and their management using plant extracts and essential oils from selected plants. Isolation of *A. solani* and *P. infestans* was done from infected tomato plant samples collected from Mwea, for morphological and molecular characterization. Crude extracts and essential oils of garlic, ginger, Mexican marigold and tick berry were tested i*n-vitro* to determine their efficacy against the two pathogens. *Alternaria solani* and *P. infestans* were cultured in Potato Dextrose Agar and V8 agar respectively after amending the media with different plant extracts and essential oils. Pathogen radial growth was monitored per treatment over time. Tomato plants were grown in the greenhouse and inoculated with spores of cultured isolates of *A. solani* and *P. infestans.* The inoculated plants were sprayed with essential oils and ridomil®synthetic fungicide as a control and disease development was monitored. Data collected was subjected to analysis of variance to test for statistical significance among treatments. Means were separated using Students Newman Keuls test at 95% level of confidence. Molecular characterization of the pathogens showed that *A. solani* had high genetic variation, while *P. infestans* showed low genetic diversity. Characterization of the essential oil compounds revealed that the test plants had many compounds and terpenes were the majority. The *in-vitro* experiment revealed that both the crude extracts and the essential oils were fruitful in suppressing the growth of the test pathogens. However essential oils were more effective than the crude extracts ($p<0.05$). Among the crude extracts, garlic was the most effective biocontrol. Ginger and garlic essential oils had similar effect to Ridomil® synthetic fungicide (Metalaxyl-M and S-isomer, Mancozeb) which showed 100% pathogen growth inhibition. In the greenhouse experiment, essential oils and Ridomil®synthetic fungicide did not differ significantly ($p>0.05$) in lowering disease severity. The tomato plants treated with Mexican marigold had the highest plant height (78.208±1.28) and also the highest number of leaves (20.5±0.93) in plants inoculated with *P. infestans*. The treatment also exhibited the highest fruit yields. The effects of other treatments did not vary significantly ($p>0.05$). Essential oils used in the greenhouse experiment had similar effects to Ridomil fungicide and therefore can be tapped for their antimicrobial efficacy. This study concluded that there exists genetic variation within *A. solani* than in *P. infestans* in Mwea, Kenya. The source of variation within them needs to be studied. This study recommends that plant extracts and essential oils from ginger and garlic be used as bio-pesticides in management of early and late blight diseases in tomato as they portrayed similar efficacy to ridomil synthetic fungicide. Simpler and cheaper methods of essential oil extraction can be explored to make the oils available for use as pesticides.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum* L.) is the second most important crop in the world after potato in terms of cultivation and consumption (Costa and Heuvelink, 2018). It belongs to solanaceae family, which comprises of perennial herbaceous plants that are short lived (FAOSTAT, 2009). The crop was introduced to Europe in the 16th Century from South America where it had originated from and later in the early 1900, introduced to East Africa by colonial settlers (Aneta *et al*., 2015). Kenya is among Africa's major producers of tomatoes and is listed 6th in Africa with a total production of 410,033 tonnes (Willis *et al*., 2018). The crop is among the most demanded high value vegetable crops popularly grown for its nutritive consumable fruits (Ewulo *et al*., 2008; Costa and Heuvelink, 2018).

Tomato demand in Kenya is exorbitant throughout the year, thus providing a ready market (Masinde *et al*., 2011). The crop is a major source of revenue for low income households and medium scale commercial farmers (Willis *et al*., 2018). Tomato fruits are important sources of vitamins and minerals for good health. The crop contains calcium, potassium, iron, magnesium, zinc, and vitamins A, B, C and E (Ahmed and Singh, 2005). Tomato fruit is a healthy food which is cholesterol free, low fat content and a good source of fibre and proteins (Joanne and Beate, 2012). The fruit also has antioxidants such as carotenoid and lycopene that have attracted interest because of their role in lowering heart diseases, preventing cancer and muscle degeneration (Ochilo *et al*., 2019). Tomato juice is used as cure for liver and intestinal disorders (Joanne and Beate, 2012). In Kenya, tomato is important in supplementing nutritional and domestic food necessity, foreign exchange earnings, creation of employment and income generation (Sigei *et al*., 2014). Tomato production in Kenya accounts for 14% of the total vegetable produce and 6.72% of the entire horticultural production (GoK, 2012). The crop is produced either in greenhouses or open field. The latter accounts for 95% of the total production in Kenya while the former accounts for 5% of the total production (Wachira *et al*., 2014).

The tomato fruits and their processed products are sold both in international and local markets such as supermarkets, hotels and the open air markets (Mungai *et al*., 2000). Kirinyaga County is the main tomato production region in Kenya producing 13.7% of the total produce followed by Kajiado 9.1% among other counties (HCDA, 2016). The crop grows best in warm environment with an altitude ranging between 0 to 2100 m above sea level. The rainfall requirements range between 760 mm to 1300 mm with soils that are fertile and well drained, with high organic matter content and a pH range of between 5 – 7 (Okiror *et al*., 2017).

Plant diseases are some of the production constraints leading to yield and quality losses. In developing countries, over 80 million people do not have enough food and more than 10% of the food is lost due to plant diseases (FAOSTAT, 2017). Tomatoes are prone to pests and diseases from germination to harvesting stage. The common diseases are caused by viruses, fungi, nematodes and bacteria (Mark *et al*., 2006). In Kenya, tomato is vulnerable to infection by early and late blight diseases with *Alternaria solani* and *Phytophthora infestans* as the causal agents (Islam *et al.,* 2013). These diseases are among the top constraints of tomato production responsible for approximately 96% of all the pre-harvest tomato losses (Lengai *et al*., 2017). It is unknown whether *Alternaria solani* and *Phytophthora infestans* have inter and intra variations among themselves. Lack of this knowledge hamper development of effective management of these two pathogens in tomato.

Tomato plants can be infected by the early blight fungus at any stage of growth but becomes more severe at fruit set (Sallam *et al*., 2011). It causes unendurable destruction of all stages of tomato plant growth and is characterized by stem collar rot, leaf blight and fruit lesions (Lengai *et al*., 2017). Collar rot occurs when the fungus causes lesions on young tomato seedlings and eventually girdle the stem completely resulting in reduced plant vigour or death (Kemmitt, 2012). The fungus also attacks the tomato fruits at the fruit stalk and cause large sunken areas with concentric rings with a black velvety appearance on the fruit (Junior *et al*., 2011). Infected stems and petioles collapse while the fruits become greasy and decay and fall off the plant (Alexandrov, 2011). *Alternaria solani* has the ability to

survive in diseased debris of plants and in the soil for a long period of time in the absence of the host plants (Verma and Verma, 2010). The pathogen also infects other cultivated plants such as potato, pepper, egg plants and weed hosts such as black night shade and horse nettle (Peralta *et al*., 2005). *Alternaria solani* exhibit high genetic variation between isolates from tomato and potato plants in different countries (Kumar *et al*., 2008). The current study hypothesize that the pathogen does not exhibit genetic variation in tomato, cultivated at Mwea thus the reason why this study was conducted.

On the other hand, *Phytophthora infestans* is an oomycete that is closely related to fungi and causes the late blight disease of tomato or potato where it destroys the infected leaves and stems during the plant growth (Alexandrov, 2011). It can cause mass destruction of all plants growing in the field in a period of a week or two (Agrios, 2005). These pathogens are spread to the susceptible tomato plants by wind, water, weeds of leguminosae family and volunteer tomato plants (Goufo *et al*., 2008). The establishment of the disease on susceptible tomato plants is favoured by cool moist weather with relative humidity of 100% and temperature ranging between 15-21°C (Scot, 2008). Molecular characterization is a tool of identification of closely related pathogen species. Molecular identification of pathogen species is necessary for the purpose of developing management strategies that are effective in controlling diseases.

Although *P. infestans* undergoes frequent genetic changes (Shattock 1976), the pathogen portrays low genetic diversity and its distribution and population structure is influenced by host preference (Adler *et al*., 2004). The current study hypothesize that the pathogen does not exhibit genetic variation in tomato cultivated in Mwea, thus the reason why this study was conducted. Agrochemicals play a vital role in improving crop yield thus curbing famine in the globe (Mizubuti *et al*., 2007). However, there has been major concerns regarding the negative outcome that the synthetic agrochemicals have on the environment and human health. Some pathogens such as *A. solani* have been reported to have resistant to diverse fungicides (Lengai *et al*., 2017). In Kenya, management of early and late blight diseases in tomatoes and potatoes require almost weekly application of synthetic pesticides in the most prone areas (Waiganjo *et al*., 2006). The chemicals are expensive and increases

the production cost by 20% (Mizubuti, 2005). Injudicious application of synthetic pesticides results to high chemical residue levels in the produce thus a basis for rejection in the international markets (Stangarlin *et al*., 2011; Campos *et al*., 2014).

Small scale farmers rarely use protective gear during application of synthetic chemicals and many a times, compromise their own safety by not following application instructions (Goufo *et al*., 2008). Sometimes the synthetic chemical fungicides used are not effective especially if the weather conditions are favourable for disease development (Chaerani and Voorrips, 2006). Pathogen resistance to the synthetic pesticides have also been reported (Srijita, 2015). In addition, some of the active ingredients in the synthetic pesticides are hormone disruptors and may cause mutations that result in serious health problems including carcinogenesis and infertility (Srijita, 2015). For instance, fungicides such as mancozeb® contain di-thiocarbamates which breaks down to carcinogenic compounds, causing thyroid and liver tumours and testicular effects (Novikova *et al*., 2003). Therefore, exposure to such fungicides has a long term risk of development of cancer among tomato farmers and the consumers. Consequently, consumers continue to demand for safety guarantee (Mwangi, 2013) thus pushing the consumer markets to develop stringent quality requirements with regard to pesticide use in vegetable production (Campos *et al*., 2014).

Natural products of plant origin can reduce foliar pathogen populations and control disease development. Plants have been found to have compounds that are anti-mutagenic, anti-inflammatory, anti-oxidants and antimicrobial (Miraj *et al*., 2017). Plants produce these compounds in order to protect themselves against external stress and pathogenic attacks (Chew *et al*., 2009). The knowledge about the compounds that are antimicrobial in these plants is scanty. The compounds present in the medicinal plants such as terpenes are affected by environmental factors such as geographical locations, fertility of the soil, parts of the plant used, season, time of collection, temperature and light intensity (Nikolic and Zlatkovic, 2010). The composition of the secondary metabolites varies within plant species and between plants from different geographical locations, environmental factors and genetic differences (Miraj *et al*., 2017),

The plant extracts are potential alternatives in integrated pest management programs that are environmentally safe (Bowers and Locke, 2004). Furthermore, bio-pesticides of plant origin are systemic, non-phytotoxic and easily bio-degradable (Mizubuti *et al*., 2007). New agrochemicals for plant disease control are being developed from natural plant products. Under *in vitro* and *in-vivo* set-ups, plant extracts have exhibited antimicrobial activity against fungal pathogens (Kagale *et al*., 2004). Different plant species contain natural products that are toxic to numerous fungi that are pathogenic to plants (Goussous *et al*., 2010). Natural products such as extracts, either as standardized extracts or as pure compounds, offer various opportunities for new chemical discoveries owing to the inherent chemical diversity (Cosa *et al*., 2006). Therefore, there is need to replace hazardous synthetic pesticides with safer plants-derived pesticides that are less toxic and environmental friendly. Characterization of chemical compounds of plants extracts and essential oils is necessary for the purpose of identifying active compounds with antimicrobial properties. This will go along way in supporting integrated disease management in tomato production.

1.2. Problem Statement

Human, environmental and host factors play a major role in modifying the dynamics of pests and diseases in a given set up. For example, climate change variability has been associated with emergence of new diseases and pests and sometimes complicates the available options for their control. Introduction of new plant varieties that are disease resistant puts the pathogen under high pressure to mutate thus resulting in new pathotype races of different pathogens. On the other hand, imprudent use of pesticides including fungicides may result in emergence of resistant pathogen strains. Different isolates of *Alternaria solani* and *Phytophthora infestans* that vary in virulence levels have been reported in different parts of the world under diverse climatic conditions. Such pathogen variability may be expected to occur in major tomato growing areas in Kenya such as Kirinyaga County hence the need to characterize the genetic variation of these two pathogens in the area. It is unknown if there is inter and intra genetic diversity of the two pathogens at Mwea, thus hampering development of an effective disease management strategy in tomato.

Several kinds of synthetic fungicides are usually employed to control early and late blight diseases of tomato. However, effectiveness of some of these chemicals have been reduced by climate and pathogen related factors. On the other hand, there has been increasing consumer concerns over the safety of foods produced using various chemicals and many are now demanding for organically produced food products. Further concerns have been raised by ecologists since some of these synthetic fungicides have exhibited adverse effects on the environment. In addition, desperate farmers may result to imprudent use of fungicides in an attempt to save their high value crops against diseases thus contributing more to environmental pollution. Therefore, there is need to substitute synthetic fungicides with eco-friendlier alternatives for management of the early and late blight diseases in tomato. Substantial research has been done on antimicrobial efficacy of plant products such as garlic, ginger, Mexican marigold and tick berry. However, information on the effects of crude extracts and essential oils of these products on management of late and early blight of tomato is still limited, hence the need for this study.

1.3 Justification of the Study

Kirinyaga County is a hub of tomato farming in Kenya and this enterprise supports the livelihood of many smallholder farmers in the area. However, tomato production is constrained by many pests and diseases, key among them, early and late blight of tomatoes caused by *Alternaria solani* and *Phytophthora infestans*, respectively. Effective disease management starts with a proper understanding of the pathogen behaviour and genetic diversity which assists in identification of the most sustainable management method. Health and environmental concerns raised by farmers, consumers, ecologists and other stakeholders have led to a shift from traditional methods of pest and disease management to more novel ways that are deemed to be safe, eco-friendly and more economical. Kenya is rich in indigenous flora and fauna that can be explored to develop safe products for pest and disease management. For instance, plant extracts and essential oils from ginger and tick berry contain antimicrobial compounds that are toxic to fungal microbes but safe to human beings and the environment (Nashwa, 2011). In addition, such organic fungicides are bio-degradable, user friendly, safe to the consumer and cheaper than synthetic

fungicides if locally produced from locally available plants (Nashwa, 2011). Adoption of such novel alternatives for early and late blight disease control in tomato will therefore result in quality and safe tomato fruits with no chemical residues that will attract more demand in the market thus improving farm incomes. Therefore, the current study targeted to determine whether garlic, ginger, tick berry and Mexican marigold could be exploited for control of early and late blight diseases of tomato.

1.4 Research Questions

1) Is there any genetic diversity between different isolates of *Alternaria solani* and *Phytophthora infestans* isolated from tomato farms in Mwea, Kenya?
2) What are the biochemical constituents of essential oils in ginger, garlic, Mexican marigold and tick berry?
3) Do different extraction solvents influence the efficacy of anti-microbial compounds in crude plant extracts of ginger, garlic, Mexican marigold and tick berry?
4) What is the *in-vitro* efficacy of crude extracts and essential oils from ginger, garlic, Mexican marigold and tick berry against the growth of *A. solani* and *P. infestans*?
5) What is the *in-vivo* efficacy of crude extracts and essential oils from selected test plants against early and late blight of tomato?

1.5 Research Objectives

1.5.1 General Objective

To assess the genetic diversity of *Alternaria solani* and *Phytophthora infestans* in Kirinyaga County and their management using plant extracts and essential oils from selected plants.

1.5.2 Specific Objectives

1) To assess the genetic diversity between different isolates of *Alternaria solani* and *Phytophthora infestans* isolated from tomato farms in Mwea, Kirinyaga County.
2) To characterize the biochemical constituents of essential oils of ginger, garlic, Mexican marigold and tick berry.

3) To determine the effects of different extraction solvents on the anti-microbial efficacy of crude extracts from ginger, garlic, Mexican marigold and tick berry.
4) To determine the *in-vitro* efficacy of crude extracts and essential oils from ginger, garlic, Mexican marigold and tick berry against the growth of *A. solani* and *P. infestans*.
5) To evaluate the *in-vivo* efficacy of crude extracts and essential oils from selected test plants against early and late blight of tomato.

CHAPTER TWO
LITERATURE REVIEW

2.1 Botanical Description of Tomato

Tomato (*Solanum lycopersicum* L.) is an annual climbing fruit vegetable that grows into branched bush or vines with yellow flowers and leaves that are compound (Piscitelli *et al*., 2017). The fruit is a fleshy berry that is oblate to globular in shape with many pear-shaped seeds and the mature fruits are red in colour (Akida *et al*., 2015). Leaves are spirally arranged and the flowers are regular and bisexual. The plant's growth habits ranges between erect and prostrate and it is self-pollinated (Geletaw and Meskerm, 2020). The stem of a tomato plant is solid, coarsely hairy and glandular. The plant has a taproot system with dense adventitious and lateral roots (Akida *et al*., 2015). Tomato plants are described as indeterminate or determinate types. Determinate tomatoes grow like a bush to a height of about 0.9 to 1.5 metres, set fruits and then decline (Masinde *et al*., 2011). Most of the early maturing varieties are determinate types. The indeterminate types have vines that continue to grow and therefore need to be supported in order to avoid their fruits being in contact with the soil which can cause rots and other diseases (Babu *et al*., 2000).

2.2 Origin and Importance of Tomato

The crop was introduced to East Africa in the early 1900 by colonial settlers from Europe but having originated from South America (Wamache, 2005). Tomatoes are globally among the most valuable vegetable crops in terms of production (Naika *et al*., 2005; Ravelo-Perez *et al*., 2008). The crop is one of the most sought fresh market vegetable (AVRDC, 2006), which is an indication of its importance as a major food crop. In Kenya, tomato is among the most vital horticultural crops and among the leading processed vegetable (Masinde *et al*., 2011). The crop is mostly cultivated by small scale farmers in various arable lands of Kenya (Waiganjo *et al*., 2006) and is a major source of income and employment for low income rural households (Wachira *et al*., 2014). Demand for tomatoes remains high throughout the year, thus providing a ready market (Masinde *et al*., 2011). Tomato fruit is a stimulant for the kidneys and cleans off toxins from the blood stream (Tijjani *et al*., 2014). The fruit is cooked as a vegetable or consumed as a salad and in other processed forms. Tomato juice is used to treat intestinal and liver disorders (Wamache,

2005). Lycopene in tomato fruit reduces incidences of prostate cancer, heart and age-related diseases (Tijjani *et al*., 2014). The crop is rich in nutrients such as iron, calcium, phosphorus, vitamins A, B and C (Asante *et al*., 2013). The nutrient composition of tomato is as shown in Table 2.1.

Table 2.1: Nutrient value of tomato in fresh fruit ($100gm^{-1}$)

Proximate Compounds		
Constituents	**%**	**Value**
Carbohydrates	3.0%	3.90 g
Energy	1.0%	18 Kcal
Protein	1.6%	0.90 g
Total Fat	0.7%	0.20 g
Dietary Fibre	3.0%	1.20 g
Cholesterol	0.0%	0.00 mg
Minerals and Vitamins		
Calcium	1.0%	10.0 mg
Iron	4.0%	0.30 mg
Magnesium	3.0%	11 mg
Manganese	6.5%	0.15 mg
Zinc	1.5%	0.17 mg
Phosphorus	3.0%	24 mg
Vitamin A	28.0%	833 mg
Vitamin C	21.5%	13 mg
Vitamin E	4.0%	0.54 mg
Vitamin K	6.5%	7.9 µg
Vitamin B1 (Thiamine)	3.0%	0.037 mg
Vitamin B9 (Folate)	4.0%	15.0 µg

(Source: USDA, 2008)

In Kenya, tomato growing contributes 14% of the vegetable produce and 6.72% of the entire horticultural crops (GoK, 2012). Tomato production in Kenya is ranked second in economic importance among the vegetables after Brassicas (Onyambus *et al*., 2011) and is mainly grown in the rural areas by small scale farmers (Ssejjemba, 2008). Kenya is rated 6th in tomato production in Africa with a total production of approximately 397,007 tons (FAOSTAT, 2012). Tomato growing is mostly done on open fields and also under greenhouse conditions. About 95% of the production is done in the open field while 5% is under greenhouse technology (Wachira *et al*., 2014). According to HCDA (2016), Kirinyaga County is the major tomato producing area in Kenya producing 13.7 % of the

total produce. Other major counties producing tomato in Kenya include Machakos (2.6%), Nakuru (2.7%), Homa Bay (3.3%), Makueni (4.4%), Migori (4.6%), Kiambu (5.2%), Bungoma (5.5%), Meru (5.6%), Taita Taveta (6.9%) and Kajiado (9.1%) (Table 2.2).

Table 2.2: Analysis of Tomato Production in Kenya per County

Counties	**Share by Quantity**	**Quantity (Tonnes)**	**Value (Million Kshs)**	**Areas (Ha)**
Machakos	2.6 %	10,240	357	314
Nakuru	2.7 %	10,990	257	580
Homa Bay	3.3 %	13,120	638	803
Makueni	4.4 %	17,552	682	408
Migori	4.6 %	18,429	910	1,068
Kiambu	5.2 %	20,972	884	930
Bungoma	5.5 %	21,720	887	1,022
Meru	5.6 %	22,214	468	420
TaitaTaveta	6.9 %	27,400	959	548
Kajiado	9.1 %	36,460	990	1,551
Kirinyanga	13.7 %	54,524	1,070	1,978
Total	**100%**	**397,007**	**12,840**	**18,613**

Source: HCDA (2016)

Tomato crop grows best in a warm climate with an altitude range of 0 to 2100 m above sea level with rainfall requirements varying between 760 mm to 1300 mm. The crop requires well drained fertile soils with high organic matter content and a pH range of 5 – 7 (Okiror *et al*., 2017). Tomato plants can exist in diverse temperature ranges, but extremes below 10°C and above 38°C causes damage of plant tissues. The optimal diurnal temperatures ranges from 20-27°C during the day and 15-17°C during the night. The crop requires full sun most of the day for fruit formation (Akida *et al*., 2015) but higher temperatures above 28°C during the day at the time of flowering causes pollen sterility (Rice *et al*., 1994). In case of water shortage and long dry periods, flowers and buds drop and fruits usually split (Rice and Rice, 2000). High temperatures and reduced humidity in semi-arid areas of Kenya leads to high fruit sets and yields in tomatoes (Wachira *et al*., 2014).

Tomato plants are extremely sensitive to abiotic stresses such as excessive moisture, salinity, extreme temperature, drought, polluted environment and biotic stresses such as diseases (Mark *et al*., 2006). There are several fungal diseases that lower the yield of tomato

such as *Fusarium* wilt (*Fusarium oxysporum f. sp. lycopersi*), powdery mildew (*Oidiopsis taurica*), damping off (*Pythium spp*.), late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), collar rot (*Sclerotium rolfsii*) and septoria leaf spot (*Septoria lycopersici*). One of the most common disease of tomato in the world is the early blight caused by *Alternaria solani* (Selim, 2015). According to Nikam *et al*. (2015), the most catastrophic disease was early blight causing losses under field conditions and post-harvest stages resulting to 50-86% reduction in fruit yield. Tomato fruit yield losses of 0.75 -0.77 tons/ha were reported with increase in early blight disease severity (Saha and Das, 2012).

2.3 Pathogens Causing Early and Late Blight Diseases of Tomato

2.3.1 *Alternaria solani*

Alternaria solani causes early blight disease in tomato and the pathogen belongs to the phylum Ascomycota, class Deutromycetes and order Moniliales (Jones and Grout, 1986). The pathogen is a soil inhabiting fungus and spreads to host plants through air and rain splash (Agrios, 2005). It is among the large-spored group of the genus *Alternaria* which is distinguished by separate conidia borne singly on simple conidiophores. Spores are elongated, muriform, beaked, septate and dark coloured. The mycelia are branched and septate (Singh *et al*., 2014). The fungus survives as parasitic and as a saprophytic organism (Singh, 1987). Early blight disease was first identified on potato and the causative pathogen was named *Macrosporium solani*. It was later classified under the genus *Alternaria* based on the spore development in chains in a culture medium (Jones and Grout, 1897). Heavy rainfall, high humidity and temperatures ranging between 24-29°C creates a conducive environment for disease development in tomatoes and can result to the plants being absolutely defoliated (Peralta *et al*., 2005).

When the conditions are favourable, the conidia germinate and form one or more germ tubes which penetrate the epidermal cell of the host through the stomata or directly by means of appressoria or wounds by hyphal growth (Agrios, 2005). The pathogen colonizes the host by degrading the cell walls of the host by producing toxins that destroy the cells of the host therefore enabling them to acquire nutrients from the plant cells (Rotem, 1994). The toxins such as alternaric acid, altersolanol, macrosporin and zinniol act on the

protoplasm of the host and disrupt physiological processes that sustain the health of the plants (Agrios, 2005). The pathogen can survive for more than a decade in the soil, seed or in plant residues at optimum temperature (Rotem, 1998). The pathogen causes early blight disease on tomato plant foliage. It infects all the above ground parts of the tomato plant at all stages of the plant growth and development (Verma and Verma, 2010) and can cause 79% crop loss (Chaerani and Voorrips, 2006). Quantity losses to early blight disease on tomato amounts to 2.15% in resistant varieties and 42.75% in susceptible varieties (Meitei *et al*., 2012).

After penetration, lesions become visible after 2-3 days and begins to produce spores 3 - 5 days later (Sherf and MacNab, 1986). The fungus attacks the tomato fruit at the stem-end resulting to a black velvety appearance and sunken areas with concentric rings (Junior *et al*., 2011). Infected ripe or unripe fruits rot with semi ripe fruits being more susceptible (Blancard *et al*., 2012). Irregular brown/black spots also appear on older leaves and the spots enlarge forming lesions that can cause complete leaf fall with symptoms progressing from lower leaves to the upper leaves. This exposes the fruits to sunscald resulting to reduced fruit yield (Ashour, 2009). The pathogen can attack any stage of plant growth but it's more severe during the fruit set resulting to increase in fruit rot (Junior *et al*., 2011). Ripe tomato fruits are more susceptible to fruit rot than unripe ones (Chaurasia *et al*., 2013). The infection begins at the calyx end forming brown leathery areas with concentric rings and this continues to cause wounds and cracks on the fruits (Datar and Mayee, 1981). Once the disease establishes on the crop it becomes difficult to control (Smith and Kotcon, 2002).

Early blight was reported to affect older leaves more than younger leaves because of low sugar content on the older leaves of the plant (Rotem, 1994) and this is considered a predisposing factor to the disease. Late in the season the leaves of maturing plants are more prone to the disease due to translocation of the sugars that they manufacture to the fruits that are ripening. More glucose level inhibits cell wall degrading enzymes by *A. solani* (Sands and Lukens, 1974). Young tomato leaves also produce high concentrations of glycoalkaloids namely solanine, solanidine and chaconine which are effective in inhibiting *A. solani* growth compared to old leaves where the production of glycoalkaloids declines

with the age of the leaves (Sinden, 1972). Tomato plants with high fruit foliage ratio are more prone to early blight (Chaerani and Voorrips, 2006).

Late maturing tomato cultivars are less susceptible to early blight than the early maturing varieties. This is because the late maturing varieties are the indeterminate types that have vine type growth habit and continue to produce new foliage which in turn produce sugars and glycoalkaloids that inhibit the growth of *A. solani* on the leaf surface (Chaerani and Voorrips, 2006). Production of sugars and glycoalkaloids in early maturing tomato varieties declines with age and therefore become more susceptible as they age (Rotem, 1994). Late maturing varieties have better resistance because young leaves that produce glucose and glycoalkaloids are present throughout the growing season (Johanson and Thurston, 1990). High phenolic content in the leaves and stems of tomato plants influence the plant's early blight resistance (Foolad, 2002). The tannin content has been reported to fluctuate as the tomato plant mature, but the tannin content is at maximum by 10^{th} week in stems and 14^{th} week in leaves (Chaerani and Voorrips, 2006). Control of *Alternaria solani* can be through use of drip irrigation, crop rotation, adequate soil fertility and use of fungicides (Yazici *et al*., 2011). However, the fungus has low sensitivity to fungicides because it produces melanin which enhances survival and offer the pathogen competitive abilities under certain environmental conditions (McDonald and Linde, 2002).

2.3.2 *Phytophthora infestans*

Phytophthora infestans causes late blight disease in tomatoes and other solanaceous plants (Mizibuti and Fry, 2006). The genus name *Phytophthora* is coined from two Greek words; 'Phyto' that meaning plant and 'phthora' meaning destroyer (Halder *et al*., 2006). The pathogen results to massive destruction on tomatoes (Judelson and Blanco, 2005). *P. infestans* belongs to the kingdom: Chromista, phylum; Heterokontophyta, class: Oomycota, order: Peronosporales and family: Pythiaceae (Sogin and Silberman, 1998). *P. infestans* is diploid, obligate, heterothallic and biotrophic oomycete, whose asexual lifecycle is characterized by alternating phases of sporangia germination, hyphal growth and sporulation (Nowicki and Marcin, 2013). Morphologically, the pathogen is similar to fungi but phylogenetically related to brown algae or diatoms. The pathogen is sometimes

referred to as water mould because of its special adaptations to water habitat (Lamour *et al*., 2007). The pathogen's cell wall is made of cellulose and other glucans unlike fungi whose cell walls are made of chitin (Avila-Adame *et al*., 2006). The genus *Phytophthora* has no ability to synthesize thiamine and sterol and therefore acquires these essential compounds from the host plant (Nowicki, 2013).

The mycelia of *Phytophthora* is coenocytic, multinucleate, and aseptate although the cross walls do not form in old cultures (Drenth *et al*., 1994). Sporangia are borne singly on the branch tips of the alternately branched sporangiophore (Fry, 2008). Sporangium is hyaline, lemon shaped with a papilla at the distal end (Aylor, 2003). Sporangia are released from the sporangiophores by twisting and popping of the sporangiophore and disseminated by air currents (Drenth *et al*., 1995). The zoospore formation within the sporangia is temperature dependent. Vegetative spores and mycelia can exist and remain infectious in plant debris for not less than one week and continue to cause infections when they are in contact with susceptible plant tissues (Fry, 2008). The pathogen can grow on selective culture media such as rye agar, lima bean agar, pea agar, corn seed agar and V-8 agar (Avila-Adame *et al*., 2006). In the culture, *P. infestans* is slow growing and the mycelia are white and fluffy although some isolates have lumpy appearance (Nowicki, 2013).

The late blight disease has the infection phase and a necrotic phase which induces host cell death (Dodds and Rathjen, 2010). The disease is characterized by brown or black lesions on leaves, stems and fruits that appear water soaked or have chlorotic borders that later enlarge and become necrotic (Schumann and Arcy, 2000). The younger and more succulent tissues are affected first followed by older leaves (Fry *et al*., 2001). Late blight disease can cause severe injury on both green and ripe tomato fruits respectively. The fruit stalk is infected first because the spores tend to inflict the top of the fruit first and the lesions on the fruit may be covered with white to grey mouldy growth of sporangia and sporangiophores when the humidity is high (Fry *et al*., 2001). The sporangia contain biflagellate zoospores with tinsel-type and whiplash-type flagella which can swim but can also lose the flagella and produce a germ tube capable of penetrating the host tissue (Becktell *et al*., 2005). *P. infestans* also infects other wild and cultivated species of solanum

such as Irish potato, pepper, horse nettle, African night shade and eggplant (Widmark *et al*., 2007). The pathogen can also give rise to oospores which are thick walled sexual spores with ability to survive in the soil in the absence of the host plants (Majid *et al*., 2008). According to Cohen *et al*. (1997), tomatoes support more oospore production than potatoes.

The sexual cycle of the pathogen occurs when mating type A1 and A2 meet and release hormones that trigger the formation of oospores (Judelson and Blanco, 2005). For the production of oospores, both the A1 and A2 mating types are needed. However, the presence of the two types is not a guarantee of sexual reproduction since some levels of incompatibility exists (Mizubuti *et al*., 2007). The mating types are compatibility types differentiated by mating hormones but are not dimorphic forms. When the mycelia of A1 and A2 mating types interact, the mating hormones induce gametangial formation in the opposing mating types, initiating sexual propagation by means of oospore formation (Majid *et al*, 2008). The mycelia undergo meiosis during gametangia formation to form antheridia and oogonia that are haploid. The antheridium fuses with an oogonium during the sexual life cycle to form diploid oospore (Judelson, 1997). Sexual reproduction results to gene recombination that generates new virulent strains thus complicating the management of the disease (Gavino *et al*., 2000).

Screening of *P. infestans* genes involved in sexual reproduction has been done but their biological functions have not been exploited (Zhao *et al*., 2011). The oospores can survive outside the living host plants in harsh conditions but the sporangia are fragile, therefore need live plants to survive. Both the zoospores and the sporangia can infect plants but the zoospores are more aggressive than the latter (Mosa *et al*., 1991). The zoospores infect plants by formation of appressoria and penetration pegs. After penetrating the tissue, a vesicle forms, hyphae grow and a haustoria emerges to extract nutrients from the hostplant cells causing destruction of the plant tissues (Grenville-Briggs and Van West, 2005).

The pathogen infects all plant parts in all plant growth stages. Infected petioles and stems finally disintegrate at the point of infection resulting with death of all distal parts (Alexandrov, 2011). Infected fruits turn greasy, decay, shrivel and fall off the plant while

those that remain attached to the plant may not ripen. Infected plants become defoliated, the photosynthetic area is reduced and loss of fruits occurs (Rubin and Cohen, 2004). The mycelia of the pathogen are spread from infected plants to healthy plants by wind or water (Goufo *et al*., 2008). Worldwide, the pathogen causes crop losses varying between 3-5 billion dollars annually. *P. infestans* has effector molecules coded by avirulence genes that allow plant quick infection and colonization of the host tissues (Haldar *et al*., 2006). When the pathogen gets into the host tissues, a complex set of compounds such as cutinase, metallopeptidase and other proteins required for cell destruction and nutrient absorption are promptly activated (Lee *et al*., 2006). From infection to sporulation, it takes a period of three days and thousands of spores arise from a single lesion (Mizibuti *et al*., 2007).

Pathogenesis begins with sprouting of sporangia, zoospores or oospores. The most abundantly dispersed propagules are the sporangia and can either germinate directly by growth of a germ tube or indirectly by the release of zoospores. The zoospores and sporangia are short lived but the oospores can remain viable for very long time (Nowicki, 2013). The mode of sporangia germination is influenced by the temperature where temperature between 18^{o}C to 24^{o}C allows direct germination of the sporangia to occur while temperature below 18^{o}C allows indirect germination to take place (Majid *et al*., 2008). The pathogen penetrates plant tissues through wounds, epidermal cells and cuticle and develops specialized structures to suck nutrients from the host plants (Mizubuti *et al*., 2007). Spores are dispersed between plants by wind or rain. Disease development is favoured by cloudy conditions, relative humidity of 100% and day temperatures of 15 - 20^{o}C during the day (Scot, 2008). Stone (2014), reported that late blight losses can be rapid and difficult to control during wet cold weather. The pathogen causes huge losses in potato and tomato (Pacilly *et al*., 2016). *P. infestans* epidemics are propagated by abundant sporulation, dissemination by wind, dispersal by human transport and the potential of the pathogen to survive in plant parts either in storage or in the field (Kawchuk *et al*., 2011).

Early and late blight diseases can be managed by a combination of various practices such as use of disease free seeds, removal of volunteer solanaceae crops and weeds from the field, use of resistant varieties, destroying the infected crop residues, fallowing, crop

rotation and fungicides application (Maerere *et al*., 2010a). Excessive use of chemicals has been shown to have adverse effects to animal and human health, environmental pollution and development of resistance by the pathogens (Naing *et al*., 2013). Use of fungicides also increases the cost of production by 20% (Mizubuti, 2005). For example, it was reported that in Cameroon, tomato farmers applied 2-3 fungicidal sprays in a week to reduce the early and late blight diseases during the rainy season when plants are most susceptible (Fontem *et al*., 2004). In Indonesia, the fungicides were found to be effective in managing the late blight disease of tomato when applied at four days-interval (Fontem *et al*., 1996). In Kenya, farmers make up to 40 applications of fungicides in a season to control the early and late blight diseases in tomato (Waiganjo *et al*., 2006). For sustainable management of the disease, early and rapid detection of the pathogen in infected plants is required (Zwankhusizen *et al*., 1998).

2.3.3 Genetic Diversity of *Alternaria solani*

Alternaria solani exhibit high genetic variation between isolates from tomato and potato plants in different countries (Kumar *et al*., 2008). The current study hypothesize that the pathogen does not exhibit genetic variation in tomato, cultivated at Mwea thus the reason why this study was conducted. *A. solani* isolates from different host plant species vary in physiology, genetic diversity and aggressiveness when inoculated in tomato plants (Scheuermann *et al*. 2004). A study conducted by Castro *et al.* (2000) using seven isolates of *A. solani* on 14 tomato genotypes revealed that all the fungal isolates possessed different degrees of virulence on the genotypes, indicating existence of high variation within the fungus. Stammler *et al*. (2014) analysed the virulence of *Alternaria alternata* and *A. solani* on potatoes and tomatoes and reported that *A. solani* had a higher virulence than *A. alternata*. According to Alhussaen (2012), *A. solani* varies in terms of morphological and physiological characteristics such as size of mycelia, conidia and presence of transverse and longitudinal septa. Perez and Martinez (1995) observed variation among four isolates of *A. solani* in regard to morphological characters mainly colony texture, mycelia colour, colony growth, pigmentation, colony texture, colony diameter and conidia size. The current study was set to confirm whether *A. solani* has both genetical and morphological diversity. Hubballi *et al*. (2010) also reported that substrate and colony colour, topography,

pigmentation, margin, zonation, diameter of the colony (mm) and sporulation varied among the isolates.

Different countries have used different methods to study genetic diversity of *A. solani*. For example, in the United States, Cuba, Brazil, South Africa, Turkey, China, Russia Greece and Canada, genetic diversity of *A. Solani* was based on vegetative compatibility groups and molecular markers (Van der Waals *et al*. 2004). Classical markers were used in Brazil, amplified fragment length polymorphisms (AFLPs) have been used in Turkey and microsatellites markers used in South Africa (Van der Waals *et al*., 2004). *A. solani* isolates have been shown to cluster according to country, indicating that fungal isolates from the same country may not be distinctly separated by geographical origin (Martinez *et al*., 2004). This could be attributed to dispersal of the air-bone spores within a short distance and movement of plant materials within the country (Van der Waals *et al*., 2001). Fungal isolates from tomato and potato plants clustered in accordance to their host plants based on AFLP and RAPD markers suggesting the pathogen-host specialization (Martinez *et al*., 2004). In Europe, *A. solani* carries two types of mitochondrial DNA referred to as genotype 1 (GI) and genotype 2 (GII) (Leiminger *et al*., 2014). In United States, *A. Solani* isolates are resistance to fungicides resulting to significant losses in the greenhouses (Pasche *et al*., 2004). Due to high genetic variability and high degree of gene flow within countries, there are no reports of potato cultivars resistant to the disease (Vander Waals *et al*., 2004).

2.3.4 Genetic Diversity of *Phytophthora infestans*

Mitochondria and the DNA in the nucleus of *P. infestans* have been used to evaluate its population structure and evolutionary history and there are two mating types that co-exist, can reproduce and survive sexually (Fry, 2008). Although *P. infestans* undergoes frequent genetic changes (Shattock 1976), the pathogen portrays low genetic diversity and its distribution and population structure is influenced by host preference (Adler *et al*., 2004). The current study hypothesize that the pathogen does not exhibit genetic variation in tomato, cultivated at Mwea, thus the reason why this study was conducted. The current study was set to confirm whether *P. infestans* has morphological and genetic diversity. Each host group of *P. infestans* is associated to different clonal lineage: tomato with US-

1, potato with EC-1, wild solanaceous species with EC-2, and *S. betaceum* EC-3 (Adler *et al*., 2004). *P. infestans* has been classified into four main groups based on mitochondrial haplotypes: Ia, Ib, IIa, IIb (Gavino and Fry, 2002) and 1c (Gomez-Alpizar *et al*., 2008). The EC-2 and EC-3 lineages has been associated to the Ia haplotype, the US-1 lineage to the Ib haplotype; the US-6 to the IIb haplotype, the EC-1 to the IIa haplotype (Gavino and Fry, 2002) and EC-2 has also been related to haplotype IC and correlated with new species, *P. andina* (Gomez-Alpizar *et al*., 2008).

2.4 PCR Amplification of ITS Regions

A number of probes have been designed by many studies for the identification of fungal DNA by the hybridization procedure, but PCR is the most sensitive and widely used technique for the identification of fungi and is also best suited for use with clinical samples in which DNA is poorly available. Ferrer *et al*. (2001) used primers ITS1, ITS4, and ITS86 to identify fungal strains by nested PCR. Kumeda and Asao (1996) also used PCR with primers ITS1 and ITS4 followed by SSCP analysis to identify fungi pathogenic to plants. Besides these, other primer pairs have been designed for the identification of fungi (Toth *et al*., 2001). To analyze the variations in nucleotide sequences, direct sequencing of the amplified product, restriction fragment length polymorphism analysis (Toth *et al*., 2001), temperature gradient gel electrophoresis, denaturing gradient gel electrophoresis, amplified rRNA gene restriction analysis for the ribosomal gene, and SSCP analysis (Ferrer *et al*., 2001) are being used by various researchers. In the present study the ITS PCR technique was used for the successful identification of fungi.

2.5 Medicinal Plants with Pesticidal Effects

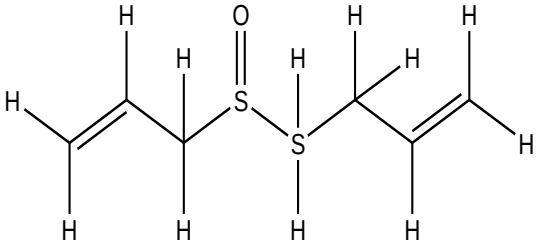
2.5.1 Garlic (*Allium sativum*)

Allium sativum L., is classified in the onion family Alliaceae and it is the second most widely used *Allium* after the onion (Tesfaye and Mengesha, 2015). It is closely related to the leek, onion, chive, rakkyo and shallot (Block, 2010). The plant is hardy thus not easily infected by pests and diseases. Garlic is an erect herb with an erect flower stock and grows to a height of about three feet. It has adventitious roots that are condensed and a flattened stem with broad leaves that are kidney shaped (Bowers and Locke, 2004). The crop does well in soils that are loose, fertile and well drained. They grow close together but leaving

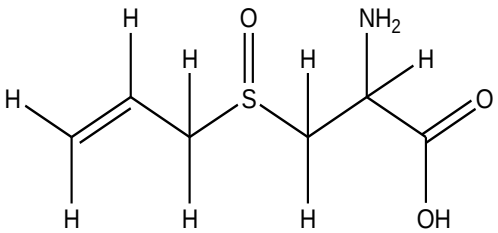
enough space for expansion of bulbs at maturity stage (Kamenetsky and Rabinowitch, 2006). Garlic varies in shape, colour, size and number of cloves per bulb depending on the variety (Fritsch and Friesen, 2002). The bulbs are covered with a transparent covering and are consumed as a vegetable and spice owing to its medicinal purposes (Stavelikova, 2008).

Plate 2.1: Garlic bulbs used in the study

Garlic has been used all over the world for both medicinal and culinary purposes (McGee, 2004) and its production worldwide was about 40 million metric tons in 2018 (FAOSTAT, 2018). Garlic bulbs have been used as medicine for millennia because of their organosulfur compounds that inhibit microbial pathogen infections (Obagwu and Korsten, 2003). It has a broad range of antibacterial and antifungal activities which is attributed to the presence of sulphur-containing compounds that are in high concentrations in the essential oil such as diallylsulphide, diallyldisulphide, diallyltrisulphide, allyl propyl disulphide, sulphur dioxide and diallyltetrasulphide (Amagase, 2006; Khadri *et al*., 2010). According to Verma *et al*. (2008), allicin (12) (diallyl thiosulphinate) in garlic is formed as a result a reaction between enzyme allinase and alliin (11) and this occurs when garlic is crushed or damaged.



11



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Garlic oil contains Alliin which is odourless and can easily be converted to Allicin through a reaction that occurs between alliin and enzyme allinase, found naturally in garlic (Lawson and Hunsaker, 2018). Allicin is also known as dially thiosulphinate and it is a powerful antibiotic and antifungal compound which gives garlic a strong pungent odour and flavour (Pedrazza-Cheverri *et al*., 2007). Dially disulphide is found in garlic oil but has weaker antifungal and antibacterial properties (Dixit *et al*., 2018). According to Harris *et al*. (2001) garlic oil contains 29.7% dially trisulphide, 4.4% dially tetra sulphide, 2.5% dially sulphide, 2.1% methyl allyl trisulphide and 3.7 % dially disulphide. Therefore, antimicrobial activity of garlic may be as a result of the cumulative action of sulphur and related compounds (Harris *et al*., 2001). The antifungal activity of garlic on plant pathogens has been used to control *Penicillium digitatum* and *Fusarium oxysporum f.sp. phaseoli* (Kanan and Al-Najar, 2008).

Garlic is used in food industry because of its sharp odour, bitter taste and appetizer properties and is consumed as fresh garlic, capsules, pills and extracts. It is safe when consumed in the correct dosage but consumption of high doses can cause stomach ulcers (Ayaz and Alpsoy, 2007). Chemical compounds of garlic destroy bacteria, parasites, fungi and also lowers cholesterol and glucose levels in the blood and fight cancerous cells. Allicin is found in garlic and is effective against a wide range of fungal and bacterial species (Stoll, 1998). Allicin in garlic is effective against gram negative and gram positive bacteria like *Lactobacillus casei, Helicobacter pylori* and *E. coli* (Cellin *et al*., 1996). Garlic water and fumes have been used to treat typhoid and whooping cough respectively (Ayaz and Alpsoy, 2007). Limuroa *et al*. (2002) reported that garlic extracts can be used to treat stomach ulcers and gastritis caused by *H. pylori*. Consumption of garlic is correlated with reduced cancer risks since its components prevent development of tumours in a variety of sites including breast, skin, cervix and colon (Milner, 2001). In Turkey, chopped garlic is added to raw meatball to slow down microbial growth in ground meat (Aydin *et al*., 2007). Compounds in garlic were reported to protect and strengthen the heart against circulatory diseases (Satyal *et al*., 2017).

Allicin compound found in garlic is effective against numerous pathogens both *in-vitro* and *in-vivo* (Portz *et al*., 2008). Slusarenko *et al.* (2008), reported that allicin from garlic effectively controlled *Alternaria* spp. in carrot and *Phytophthora* leaf blight of potato. Curtis *et al*. (2004) reported that garlic was effective against *P. infestans* on tomato seedlings while Bowers and Locke (2004) reported that garlic extracts inhibited mycelial growth of *Ralstonia solani* and *Fusarium solani*. Red garlic contains saponins and flavonoids that exhibit anti-bacterial properties against *Bacillus subtilis* (Locke, 2006). According to Ayazpour *et al*. (2010), garlic extracts increased mortality of *Tylenchulus semipenetrans* in laboratory conditions. Garlic extracts have direct effects on nematode populations by disrupting their food absorption, reproduction and mobility (Fadzirayi *et al*., 2010). Garlic essential oil was effective against free-living soil inhabiting nematodes that destroy crops (Block, 2010). Garlic was one of the test plants used in the current study to test if garlic crude extracts and essential oils can inhibit *A. solani* and *P. infestans* growth.

2.5.2 Ginger (*Zingiber officinale*)

Ginger is a common herb belonging to the *Zingiberaceae* family and widely used as a medicinal plant and in beverages (Afzal *et al*., 2001). The plant is a perennial creeping with a slender erect stem about 2-3 feet in height, covered with smooth leaf sheaths and rarely flowers (Markson *et al*., 2018). Ginger grows well in warm tropical climate with loam soils that are loose to allow expansion of rhizomes and requires hot and moist climate with some shading for its growth (Sinedu *et al*., 2015). The herb has an edible underground rhizome, branched and thickened to form a structure that resembles a swollen hand and a spicy lemon-like scent. The rhizome is brown in colour with buds that give rise to shoots and is consumed as medicine, delicacy or as a spice (Abdel-Aziz *et al*., 2006).

Plate 2.2: Ginger rhizomes used in the study

Ginger rhizome is highly demanded in the world markets. The rhizomes have been used in herbal medicine for treatment of diabetes, gingivitis, nervous diseases, constipation, rheumatism, catarrh, asthma, toothache and stroke (Javid *et al*., 2019). Ginger extracts have inhibitory effects against *Candida albicans* that cause candidiasis (Deboer *et al*., 2005). The anti-viral compounds in ginger rhizome relieve against common cold virus (rhinovirus). Morning sicknesses in pregnant mothers and inflammation were also treated using ginger rhizome (Javadi *et al*., 2013). Compounds such as gingerols, gingerdione, gingerdiol and shogaols alleviate flu symptoms by suppressing cough, reducing fever and pain and have sedative effects that encourage rest (Kalra *et al*., 2011). Migraine headache can be treated without any side effects by consumption of ginger rhizomes (Mustafa and Srivastava, 1990).

In industries, ginger rhizomes have been used to manufacture medicine, cosmetics and ready made foods (Kaushal *et al*., 2017). Ginger contains volatile oils, alkaloids, mucilage and phenols that have therapeutic benefits (Wang and Wang, 2005). Its pharmacological properties such as anti-oxidants, anti-inflammatory, and anti-cancer from ginger extracts are due to the presence of shogaols and gingerols (Harliansyah *et al*., 2007; Yeh *et al*., 2014). Gingerols may be modified during thermal processing or storage, to a series of homologous compounds of shogaols such as 6-, 8-, and 10-shogaol (Sang *et al*., 2009; Shao *et al*., 2010) with un-branched alkyl chains of different lengths (Masuda *et al*., 2004). The desiccated form of gingerols (shogaols), are homologous phenolic alkanones (Dugusani *et*

al., 2009). Among the shogaols, 6-shogaol has many biological effects such as antifungal, anti-oxidative and antibacterial properties both *in-vitro* and *in-vivo* (Mahady *et al*., 2003). A study carried by Bhattarai *et al*., (2001), reported that 6-gingerol can isomerize to 6-shogaol in a model system such as an acidic condition with high temperature.

Gingerols have known anti-cancer properties against gastric, colon and skin cancers (Mahady *et al*., 2003). Among the gingerols, 6-gingerol has been found to possess diverse pharmacological effects such as anti-pyretic, chemo-preventive, analgesic, anti-inflammatory, angiogenesis and anti-oxidant application (Kim *et al*., 2005). Ginger rhizomes contain anti-oxidants and anti-carcinogenic properties that prevent production of free radicals thus slowing the aging process and preventing diseases (Abdel-Aziz *et al*., 2006). Ginger rhizomes have also been shown to relieve nausea related to chemotherapy in patients receiving chemotherapy for various types of cancer (Manju *et al*., 2005). Experiments conducted in the laboratories have furnished scientific information to support the belief that ginger contains anti-inflammatory properties (Grzanna *et al*., 2005). Ginger constituents and non-steroidal anti-inflammatory drugs share pharmacological properties (Grzanna *et al*., 2005). Ginger compounds have been used to prevent coronary diseases by reducing platelet aggregation without affecting blood sugar or blood lipids (Kaushal *et al*., 2017). Ginger has been used to manage ovarian and prostate cancer (Jeong *et al*., 2009).

Ginger was also found to be effective against crop pathogens and pests (Stoilova *et al*., 2007). Extracts from ginger were found to have fungicidal properties and worked against *Fusarium* spp, *Curvularia* spp, and *Colletorichum* spp (Krishnapillai, 2007). Ginger has many biological effects including antifungal, anti-oxidative and antibacterial properties both *in vitro* and *in-vivo* (Dugasani *et al*., 2009). Hot water extracts from ginger were found to inhibit the growth of *A. niger, A. flavus* and *F. oxysporum* in culture and reduced rotting of yam tubers (Okigbo and Nmeka, 2005). The main components of the essential oils are β-bisabolene, ar-curcumene, β-sesquiphellandrene and α-zingiberene (Stoyanova *et al*., 2005). The essential oil was found to have a substantial fungicidal effect on the fungi *Aspergillus niger*, *Penicillium sp*., *Rhizopus nigricans* and *Botrytis cinerea* (Stoyanova *et al*., 2005). Various methods of extraction have been used to acquire bioactive constituents

from ginger, such as reflux, shaking at room and warm temperature (Jiang *et al*., 2005), high pressure soxhlet extraction and sonication (Wohlmuth *et al*., 2005). Ginger was one of the test plant used in the current study to test whether its crude extracts and essential oils have any antimicrobial activity against growth of *A. solani* and *P. infestans*.

2.5.3 Tickberry (*Lantana camara*)

Tickberry is a hardy, evergreen shrub with a characteristic aroma. The shrub grows up to a height of three metres, with or without minute prickles on the branches (Sharma *et al*., 2005). The stems are woody and hairy when young and develop some thorny like structures as they grow (Patel *et al*., 211). Leaves are about 2-10 cm with serrated leaf margins and the leaves grow opposite one another as network veined (Mishra, 2015). Flowers are pink, yellow or white in colour but fruits are black in colour and the plant has a tap root system with numerous lateral roots. The perennial shrub can grow in an altitude of up to 2,000 metres above sea level in temperate, sub-tropical and tropical parts of the world with rainfall of about 900 mm (Dua *et al*., 2010). The plant grows well in a wide range of habitat and on different soil types and does well in unshaded areas such as rainforest edges, wastelands and forests recovering from fire (ISSG, 2015). The plant grows besides roads, railways and canals. In most parts of Kenya, the plant has been used as live fence in the homesteads and demarcating boundaries.

Plate 2.3: Tick berry plants used in the study

Tickberry has several uses mainly in herbal medicine, as firewood and mulch (Ashish *et al*., 2011). In the field of herbal medicine, the plant has been exploited in the treatment of

measles, chicken pox, cancers, high blood pressure, tumours, ulcers, tetanus, and malaria (Lonare *et al*., 2012). Several tri-terpenoids, alkaloids, glycosides and flavonoids isolated from tickberry are known to exert a wide range of biological activities (Prasad and Purohit, 2009). Extracts from the leaves possess a larvicidal activity while extract from the flowers of the plant show insect repellent activity (Prasad and Purohit, 2009). Tickberry secretes allelochemicals in the rhizosphere which inhibit germination, growth, development or metabolism of the crops planted closer (Qasem, 2006).

Tickberry allelochemicals disrupt cell membrane permeability, ion uptake, cause damage to DNA and protein, inhibition of electron transport in both the respiratory chain and photosynthesis, alterations of some enzymatic activities and eventually lead to programmed cell death of the affected plant (Ding *et al*., 2007). Leaves of tickberry have been shown to be a good source of insecticidal activity and a rich source of bioactive molecules (Dua *et al*., 2010). In the laboratory studies, Ogendo *et al*. (2003) reported that tickberry powdered leaves at 10 % w/w, caused 90 % mortality of *Sitophilus zeamais* adults 21 days after treatment. Other documented information indicates that aqueous tickberry extracts, at 1 % w/v, caused complete feeding inhibition of first instar larvae of *P. brassicae* and also reduced the infestation of tea leaves by the tea mosquito bug (Sharma and Gupta, 2009). The tickberry plants are said to contain flavonoids, tri-terpenoids and alkaloids such as lantanine which is the substance having insecticidal action (Ogendo *et al*., 2003). The leaves of tickberry can be a promising source of a new bio-pesticide. Essential oils in tickberry are mainly mono and sesquiterpenes and the most representative constituents include farnesene derivatives, (E)-cariophylene, germacrene D (Machado *et al*., 2012) bicyclogermacrene, isocaryophyllene and valencene (Sousa *et al*., 2012).

The essential oils exhibit significant antibacterial activities against various strains of bacteria (Sousa *et al*., 2012). Essential oils of tickberry exhibited insecticidal, anti-feedants, anti-microbial and anti-helmintic properties (Singh *et al*., 2012). Leaf extracts of tickberry inhibited spore germination of *Riccia billardieri* (Chaudhary *et al*., 2007). The extracts of tickberry contained several phenolics that were identified by high performance liquid chromatography (HPLC) such as vanillic, gentisic, salicylic, caffeic, ferulic, coumarin and

many more (Yi *et al*., 2005). The leaves, stems and roots of tickberry were found to contain allelochemicals which restricted germination of *Funaria hygrometrica* and the restriction varied between plant parts, with leaves having the highest inhibition followed by the stem (Choyal and Sharma, 2011). Extracts of tickberry were found to have adverse effects on germination of mung bean seeds (Maiti *et al*., 2010).

In-vitro antibacterial activity of ethanol extracts of tickberry was investigated and was reported that the extracts restrained the growth of both gram positive and gram negative bacteria (Xavier and Arun, 2007). For example, using the disc diffusion method, notable zones of restriction against the test bacteria were reported. Essential oils of tickberry were reported to have prominent bacterial activity against all strains of bacteria tested. From the results, it was noted that gram positive bacteria were the most sensitive strains (Saikia and Sahoo, 2011). Kumarasamyraja *et al*. (2012), reported that extracts of tickberry showed antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Methanol extracts of tickberry exhibited moderate inhibition against *A. niger* but high inhibition against *E. coli* and *S. aureus* (Barsagade and Wagh, 2010). High concentration of tickberry of over 25mg/ml was needed to inhibit the growth of *Aspergillus flavus* and production of aflatoxin B1 from the fungus (Mostafa *et al*., 2011). Tickberry extracts also inhibited the growth of *Phytophthora infestans* (Maharjan *et al*., 2010).

According to Srivastava and Singh (2011), tickberry extracts at a concentration of 20 mg/ml inhibited the growth of *A. solani* with 10 mg/ml exhibiting the lowest growth inhibition. Decomposed leaves of tickberry were found to cause remarkable changes in the fungal population composition of the soil and endorhiza, favouring the fungal species that exhibited strong nematicidal and hatch inhibiting activity (Bouda *et al*., 2001). Decomposed leaves and leaf extracts of tickberry suppressed several root-infecting fungi (Shauka *et al*., 2001). Tickberry plants can prevent soil erosion and compaction and when it decomposes, the organic matter improves soil fertility. Tickberry manure applied in rice field increased tillering and fast growth resulting to higher yield (Singh and Angiras, 2005).

Tickberry leaves used as manure in sunflower farms improved chemical constituents of sunflower plants and the crop yield (Dawood *et al*., 2012). Ingestion of the leaves of tickberry by grazing animals resulted to hepatotoxicity and photosensitization in animals (Sharma and Sharma, 1989). The toxicity was due to the presence of lantadene A and lantadene B (Khan *et al*., 2003). Seven compounds were isolated from the vegetative part of tickberry and were tested for nematicidal activity against root knot nematode (*Meloidogyne incognita)* and results showed that lantanolic acid, lantoic acid and pomolic acid caused 100% mortality at 1.0% concentration after 24 hours (Begum *et al*., 2008). Camarinin, camarin, ursolic acid and lantacin caused 100% mortality of the test nematode at 1.0% concentration after 48 hours (Begum *et al*., 2008).

Premature leaves of tickberry were found to be active in biosynthesis and accumulation of secondary metabolites and had higher antioxidant activity than older leaves. Methanolic extracts from tickberry leaves occupying position 1 and 3 on the twig exhibited a higher antioxidant activity than leaves occupying position 4 and 5 (Bhakta and Ganjewala, 2009). Essential oil from tickberry leaves was documented to have insecticidal activity against mosquitoes (Dua *et al*., 2010). Ethanol and methanol extracts from flowers and leaves of tickberry showed larvicidal activity against mosquito larvae in their 3^{rd} and 4^{th} instar stages (Kumar and Maneemegalai, 2008). Tickberry extracts have been used to treat human diseases such as ulcers, asthma, measles, bilious fevers, cancer, catarrhal infections, high blood pressure, malaria, rheumatism, tetanus, eczema and atoxy of abdominal viscera (Mishra and Singh, 2009). Leaves of tickberry are chewed for the purpose of treating toothache and also used as an inhalant for headache and flu treatment (Kokwaro, 2009). Essential oils from tickberry are used for treatment of cuts, skin itches, scabies and wound infections (Ashish *et al*., 2011). Tickberry was one of the test plant used in the current study to test whether the antimicrobials reported in the plant have any effect on *A. solani* and *P. infestans* growth.

2.5.4 Mexican Marigold (*Tagetes erecta*)

Mexican marigold is an annual aromatic herbaceous plant from the Asteraceae family and genus Tagetes and a major agricultural weed in many countries (Sadia *et al*, 2013). In

pastures, the weed is problematic within East and South Africa, Australia and South America (Makang'a, 2012). The plant is known by several common names such as wild marigold, Mexican marigold, khaki bush, stinkweed and stinking roger (Randall and Kessal, 2004). The herb is strongly scented with a height of about 50-150 centimetres, erect and highly branched. Sometimes it is planted in between crops as an insect repellent because of its sharp peculiar smell (Shahzadi, 2012). The herb has a tap root system surrounded by many fibrous lateral roots forming mycorrhizal associations. The stems are woody, ridged or grooved and usually branched on the upper part (Anonymous, 2003). The leaves are about 3-30 centimetres long and 0.7 to 8.0 centimetres wide, dark green, glabrous, pinnate with 9-17 leaflets which are opposite each other with serrated leaf margins (Soule, 1993). The arrangement of flowers is in solitary clustered panicled branches with numerous flower heads that are yellowish green in colour. Flowering process is high in temperatures ranging from 14.5 to 28.6^{o}C (Wang and Chen, 2006).

Mexican marigold grows well in both dry and moist areas from sea level up to 3000 metres above sea level with loose soil and soil pH ranging from 4.3 to 6.6 and well drained (Holm *et al*., 1997). The herb is described as a weed and can grow on roadsides, waste ground, farms, vine yards and orchards (Hulina, 2008). The herb requires rainfall of about 500 mm distributed over the growing season or irrigation water sufficient for its growth (Singh *et al*., 2003). The plant grows under extensive range of climatic conditions from tropical to extreme temperate regions (Shahzadi, 2012) but prefers temperature range of between 12°C - 30°C for the production of quality oil (Bandana, *et al*., 2018). Seeds cling on the animal hair and human clothes thus dispersed to colonize knew environment away from the mother plant (Singh *et al*., 2003).

Plate 2.4: Mexican marigold plants used in the study

Mexican marigold has orange glands located under the surface of the leaves and when punctured, they produce liquor like aroma (Wanjala and Wanzala, 2016). The roots produce secretions that contain phenols, amines, flavonoids, ketones and amides that have insecticidal and nematicidal effects (Setshogo, 2005). Many Tagetes species have been found to contain terpenoids, thiophenes, flavonoids, carotenoids, phenolics among others with various biological activities such as antiplasmodial, antimicrobial and insecticidal (Gupta and Vasudeva, 2012).

Essential oil obtained from Mexican marigold leaves is reported to contain a total of forty-four constituents with major constituents being limonene, piperitone, terpinolene, (Z)-myroxide, and piperitenone (Krishna *et al*., 2004). The essential oil is also constituted of compounds such as β-phellandrene, α-pinene, d-limonene, ocimene, linalyl acetate, β-pinene, geraniol, dipentene, tagetone, nonanal, menthol and linalool (Krishna *et al*., 2004). The oil also contains flavonoids such as 7-glucoside, quercetin-3-rhamnoside, quercetin-5-glucoside, quercetin-3-glucoside, quercetin-3-methyl ether, kaempferol-7-glucoside, uercetagetin, kaempferol-3-glucoside, quercetagetrin and 6-hydroxykaempferol-7-O-glucoside, kaempferitrin, kaempferol, kaempferol-7-O-rhamnoside, and later quercetin, patulitrin and patuletin (Gounot *et al*., 2008).

The differences in chemical composition of the essential oils of Mexican marigold has been attributed to factors such as geographical location of the target plant, harvesting method, stage of growth at the harvesting time, plant parts (leaves, flowers, stems or roots) used and the climatic conditions under which the plant is growing (Chamorro *et al*., 2008). A study on essential oils of Mexican marigold aerial parts obtained from United Kingdom, South Africa and Egypt showed that the chemical composition and bio-activity of the oils varied significantly with geographical location of the plants (Senatore, 2004). Variations in essential oil chemical composition of the same plant species may also be attributed to chemotypes due to effects of temperature variation, weather conditions of the area, different types of soils, and light among other factors (Rohloff, 2003). This implies that there is a possibility that plants that are botanically identical can have oils that differ in chemical composition.

In Nigeria, Mexican marigold extract significantly suppressed soil nematode population and galls on cowpea roots thus was recommended for management against nematode pests of cowpea (Olabiyi and Oyedunmade, 2007). The essential oils containing (Z)-tagetonone and dihydrotagetone were evaluated against juveniles and eggs of a plant parasitic nematode known as *Meloidogyne incognita* and it was reported that the oils were effective on both the eggs and the juveniles of the test nematode by killing the juveniles faster (Adekunle *et al*., 2007). In South Africa, Muyima *et al*. (2013) reported Mexican marigold essential oils to have antimicrobial and worked against the gram negative bacteria (*Pseudomonasa eruginosa* and *E. coli*) and the gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The activity of the essential oil against the test bacteria increased with concentration, but gram positive bacteria were more susceptible to the essential oil than gram negative bacteria (Muyima *et al*., 2013). Essential oils containing more phenolics were said to have more antimicrobial activity (Lambert *et al*., 2001). Mexican marigold essential oil has been reported to have antifungal activity against *Botrytis cinerea* (Barkai-Golan, 2001). The plant has also been used as an ornamental for landscaping and beautification (Sadia *et al*., 2013).

Essential oils from the Mexican marigold leaves were found to be more effective against *Rhizoctonia solani, Fusarium oxysporum pisi*, *F. oxysporium lentis, Fusarium solani, Alternaria solani, Pyricularia grisea, Sclerotium rolfsii*, and *S. sclerotiorum* as compared to oils from the flowers of the same plant (Kumar *et al*., 2019). The oil from flowers was found to have the highest inhibition on *F. oxysporum pisi* and the lowest inhibition was on *P. grisea* (Saha *et al*., 2012). The oil from the leaves of Mexican marigold had the highest inhibition on *S. rolfsii* and least inhibition on *F. oxysporum lentis (*Martinez 2012). Mexican marigold essential oil has also been reported to have efficacy against cabbage aphids causing 90% mortality (Motazedian *et al*., 2014). The essential oils were also reported to have repellent and antioxidant activity against diamond back moth (Reddy *et al*., 2015). Mexican marigold essential oils containing (Z)-tagetonone, (E)- tagetonone, (Z)-tagetone, (E)-tagetone, dihydrotagetone and piperitenone had a high repellence of mosquitoes (Wanzala and Ogoma, 2013). The oils also showed inhibitory and oxidant activity on the roots of maize seedlings (Scrivanti *et al*., 2003). Some compounds such as β-pinene and α-pinene also have a strong allelopathic effect (Amri *et al*., 2013). Mexican marigold was one of the test plants used in the current study to test whether the antimicrobials reported in the plant have any effects on *A. solani* and *P. infestans* growth.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

The research was conducted in Mwea, Kirinyaga County, Kenya (Fig. 3.1). Mwea is a semi-arid area with an altitude of 1100 metres above sea level and is mainly covered by black cotton soils. The area lies under low midland agro ecological zone characterized by gentle rolling plains (Kenya County Guide, 2015). Annual rainfall ranges between 800-1250 mm and is usually received in two seasons. The area is also supplied with canal irrigation water from rivers Rupingazi, Thiba, Rwamuthumbi and Ragati (Kenya County Guide, 2015). The annual temperature ranges between 19.6 -26.3ºC. The kind of vegetation found in the area is savannah grassland and woodland. The major economic activity is agriculture. In Mwea, farmers specialize in production of food crops such as rice, maize, beans and horticultural crops for both home consumption and commercial purposes.

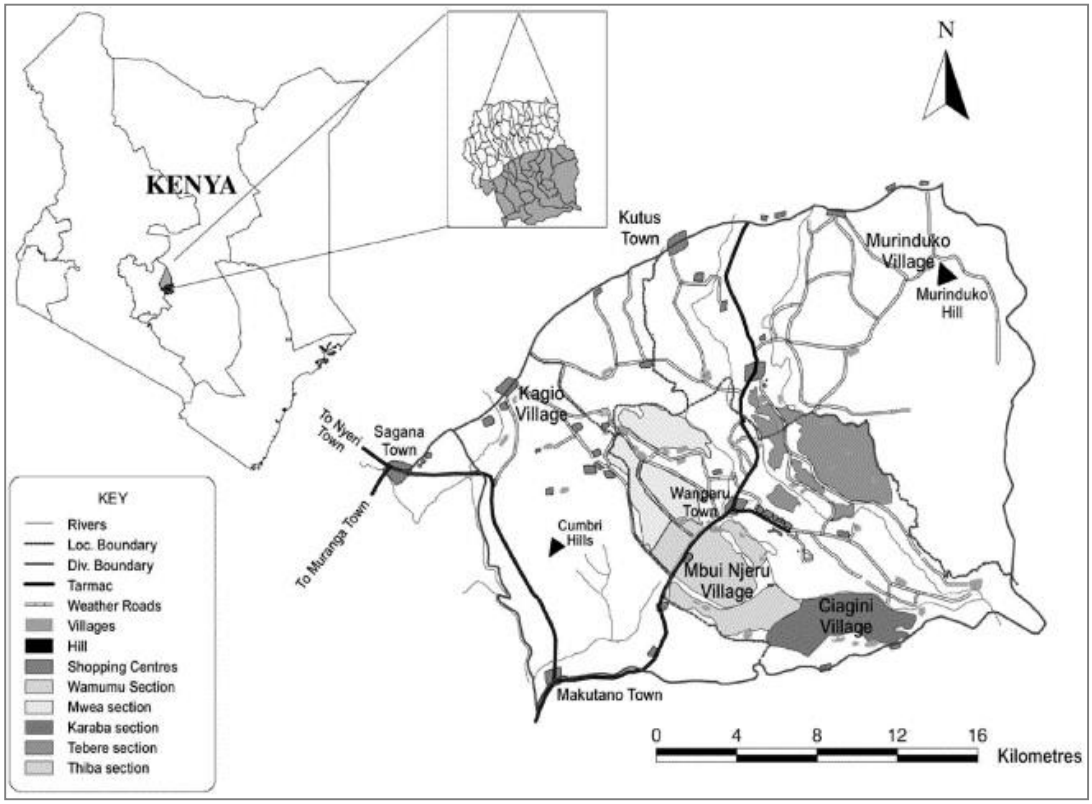

Figure 3.1: Mwea map showing the study site (Source: wikipedia maps)

3.2 Collection of Infected Tomato Plant Samples

Tomato farms in Mwea area were targeted for the collection of diseased plant samples used in this study because the area has a long history in tomato production and is a hot spot of early and late blight diseases. Four villages namely Mbui Njeru, Red Soil, Ciagini and Wang'uru were purposively sampled for sample collection because tomato production majorly takes place in these areas. Fifteen tomato producing farms in each village were randomly sampled for collection of diseased plant samples. Diseased tomato leaves bearing symptoms of early and late blights were identified by physical examination (Plate 3.1). The diseased plant materials were collected randomly from the sampled farms, placed in cool boxes and brought to Microbiology Laboratory at the University of Embu for processing and further analysis. The diseased plant samples were stored at 4ºC in a refrigerator waiting processing.

Plate 3.1: Infected tomato samples collected from farms in Mwea

3.3 Preparation of Experimental Materials

3.3.1 Isolation of Pathogens from the Infected Tomato Plant Samples

The isolation technique used was as described by Naik *et al.* (2010). Potato Dextrose Agar (PDA) and V8 agar were the standard media used to isolate the *Alternaria solani* and *Phytophthora infestans* respectively, from the unhealthy tomato plant samples. The infected tomato plant leaves bearing blight symptoms were first washed with running water and then surface sterilized for 3 minutes by dipping them into 1% Sodium Hypochlorite. Rinsing was done in three changes of sterile distilled water and sterilized blotting papers

were used to blot them dry. Infected leaf tissues of 3 mm x 3 mm size were cut using a sterilized scapel from the infected plant leaves moving from the healthy portions to the infected portion where the pathogens were suspected to be more active. The dried infected tissues having symptoms of early blight were directly plated on sterile PDA and those having symptoms of late blight were directly plated on V8 agar and then incubated at room temperature (25ºC) for 3 days in the laboratory (Plate 3.2). The pathogen colonies that formed were isolated and sub-cultured through single spore isolation by use of a single hypha separately on sterile PDA and V8 agar respectively to obtain pure culture strains for identification and molecular characterization.

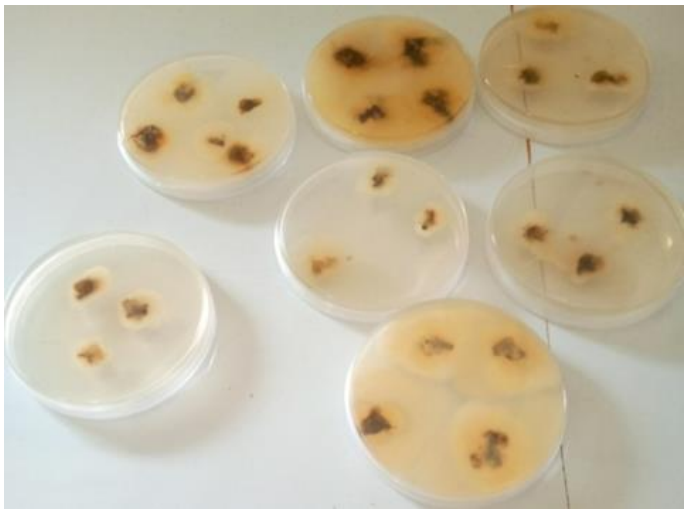

Plate 3.2: Direct plating of infected tomato leaf tissues

3.3.2 Pathogen Identification

Identification of the pathogens was done to ensure that the pathogens isolated were those that cause early and late blight diseases in tomato. Identification of pathogen isolates was done using morphological characteristics, using standard identification keys (Alexopoulos *et al*., 2002). The isolates were subjected to microscopic and colony scrutiny during which their morphological characteristics were examined and recorded. Identification was rooted on visual observations of the pathogens' growth patterns on the growth media (both front and reverse), colour of mycelia and microscopic assessment of reproductive and vegetative structures. Identification was done up to genus level and colour charts were used. The

identified pathogens (*Alternaria* and *Phytophthora*) were maintained on plates waiting pathogenicity tests, assessment of genetic diversity and bioassays.

3.3.3 Pathogenicity Test

Pathogenicity test was conducted using techniques of Okigbo *et al*. (2009) in accordance to Koch's postulates to test whether the pathogens isolated will cause the similar disease symptoms to those in the collected tomato samples. Kilele F1 tomato seeds were bought from certified seed merchants at Mwea and were sown in a screen house at the University in plug trays with peat growth medium for easy transplanting. After three weeks, the seedlings were lifted from the plug trays and transplanted into individual plastic pots filled with sterilized potting mixture of sand, soil and well decomposed manure in the ratio of 3:2:1 in the screen house. The pathogen isolates were sub-cultured for two weeks at 25ºC in an incubator before application. Preparation of spore suspension was done by adding 5 ml of sterile distilled water to a pure fungal culture in a petri-dish. Dislodging of the spores was done with a bent glass rod and the content passed through a three-layer cheese cloth to filter out the mycelia. The concentration of the spore suspension was determined using haemacytometer slide under a microscope and then adjusted to $1x10^6$ spores per millimeter with sterile distilled water and used immediately.

The newly transplanted seedlings were allowed to recover from the transplanting shock and regain active growth before being inoculated with the two different isolates while the un-inoculated seedlings acted as the control. There were two different sets of experiment for the two pathogens, *A. solani* and *P. infestans*. After disease development, infected plant samples were also collected from the tomatoes in the experiment and pathogens isolated as done earlier and compared to the first isolates from the field.

3.4 Assessment of Genetic Diversity within Pathogen Isolates

3.4.1 DNA Extraction of the Isolates

The DNA extraction process followed the modified procedure of Aamir *et al*. (2015). Isolates of *A. solani* and *P. infestans* were cultured in PDA and V8 agar respectively for five days. Sterilized wooden tooth picks were used to aseptically scrap the isolates mycelia

into separate eppendorf tubes per isolate. Extraction of total genomic DNA of the pathogen isolates was done using lysis buffer (50mM Tris pH 8.5, 20mM EDTApH 8.0 and 25% sucrose solution; 10mM Tris pH 8.5, 50mM EDTA pH 8.0 and 1% SDS) and the content in the eppendorf tubes were mixed by inversion. To the mixture, 10 µl of proteinase k (200 mg/l) was added and vortexed then incubated at 65^{o}C for 60 minutes. Equal volume of phenol-chloroform was added and the mixture was centrifuged at 13200rpm for 5 minutes at 4^{o}C. The supernatants were transferred to new tubes and their volumes were noted. One hundred and fifty (150) microlitres of sodium acetate (pH 5.2) was added to each of the supernatants. Equal volume (to the supernatant and sodium acetate) of isopropyl alcohol was added to the mixture and the content was inverted gently in order to mix. The tubes were spun at 13,200 rpm for 10 minutes and the supernatant discarded. The resulting DNA pellets were washed by adding 500 µl of 70% ethanol and then centrifuged for 1 minute at 10,000 rpm. The supernatant was discarded and DNA pellets washed again with 70% ethanol and then re-suspended in 50 µl PCR water and stored at -20^{o}C for further processing.

3.4.2 PCR Amplification and DNA Sequencing

PCR analysis was done in a 25 µl reaction mixture comprising 1 µl of genomic DNA, 0.5 µl of each of the forward and reverse primers (ITS1 and ITS4), DNA taq polymerase (0.125 µl), 2.5 mMdNTP'S (2.5 µl), 0.125 µl of premix Taq buffer and 2.5 µl of 10x dream Taq buffer ($Mgcl_2$) as described by Aamir *et al*. (2015). The final volume was topped up with 17.75µl with molecular grade water to make 25 µl. Amplification was performed in a PCR with the following cycling conditions: an initial hybridization at 95^{o}C for 2 minutes, followed by 35 cycles of denaturation at 95^{o}C for 1 minute, annealing at 54^{o}C for 1 minute, extension at 72^{o}C for 1 minute and final extension at 72^{o}C for 2 minutes. The amplicons were resolved by gel electrophoresis in 1.2% agarose gel stained with ethidium bromide (0.5 µg/ml). The DNA bands resolved on agarose gel were visualized in UV light and photographed. The sizes of the amplicon were estimated by comparing them with a commercial cleaver CLS-MDNA-100bp DNA ladder#SM0331. The ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCCTCC GCT TAT TGA TAT GC-3') primers were used to amplify the ITS region and then sequenced using the same primers used for

amplification (Pandey *et al.*, 2003). After electrophoresis of PCR products, they were cleaned. Sequencing was done in South Africa. The sequences were edited before comparison with samples in the GenBank. Nucleotide BLAST program was used to carry out sequence similarity searches to find out closely related species from the GenBank. Identification of species was made based on greater than 99% similarity between the query and reference sequence (Tamura *et al*., 2011).

3.5 Extraction and Bio-chemical analysis of the Essential oils

3.5.1 Extraction of Essential Oils from the Test Plants

The test plant materials used in this study were ginger (*Zingiber officinale*) rhizomes, garlic (*Allium sativum*) cloves, tick berry (*Lantana camara*) leaves and Mexican marigold (*Tagetes erecta*) leaves. Fresh tickberry and Mexican marigold leaves were collected from the field while ginger rhizomes and garlic bulbs were obtained from the local open air market. For extraction of essential oils, 5 kilograms each of tickberry and Mexican marigold leaves were washed with running water, rinsed three times with distilled water and then air-dried in the laboratory to evaporate the moisture content. Five (5) kilograms each of garlic cloves and ginger rhizomes were also washed with running tap water and then rinsed with distilled water three times. Garlic and ginger test materials were also air-dried in the laboratory and later peeled. All the fresh materials were stored in the laboratory under room temperature awaiting the extraction of essential oils.

Extraction of essential oils was done following a modified procedure described by Adams (2007). A vertical steam distillation unit, consisting of a biomass flask, boiling flask, hot plate, still head, condenser and receiving flask (separating funnel), was used for steam distillation of plant material. Five (5) kilograms of the fresh materials of each plant were put separately in the biomass flask and the distillation unit was switched on. A hot plate was used to heat distilled water in the boiling flask to produce steam. The steam then moved upward into the biomass flask where it caused the rapture of plant cells thus generating essential oils and other water-soluble plant compounds that were moved as vapour via the still head, condensed in the water-cooled condenser, and collected in the receiving flask (separating funnel). The receiving flask separated the lighter-than-water oils from the

heavier-than-water oils while allowing excess water-soluble compounds to be drained out and collected separately. The obtained samples were further dried to remove any water molecules by passing the oil in anhydrous sodium sulphate (Na_2SO_4). The essential oils were divided into two samples: one for analysis of their constituents and the other for bioassays and put in air tight bottles and stored in the refrigerator at a temperature of 4ºC awaiting the analysis of the chemical compounds and the bio-assays.

3.5.2 Bio-chemical Analysis of Essential Oils

Chemical analysis of essential oils followed the modified procedure described by Omolo *et al*., (2004). The HP 5890 series II Gas Chromatograph interfaced to a 5977 Mass Selective Detector (MSD) and an autosampler and controlled by HP Chemstation software was used to analyse the phytochemical compounds in the essential oils. A HP5-MS capillary column (30.0 m x 250 m x 0.25 m) film thickness was used for chromatographic separation. The column stationary phase was comprised of 5:95% diphenyl: dimethylpolysiloxane blend. The initial oven temperature for the operating Gas Chromatograph (GC) condition was set at 40ºC for 3 minutes, then programmed to 250ºC at the rate of 5ºC/minute, then kept constant at 250ºC for 40 minutes. The detector and injector temperatures were set at 250ºC and hydrogen was used as the carrier gas flowing at a rate of 1ml/minute. The mass spectrometer was operated in the electronic impact mode at 70eV. Ion source and transfer line temperature was kept at 250ºC. The mass spectra were obtained by centroid scan of the mass range from 40 to 800 atomic mass unit (amu). Hundred microlitre of each of the oil was diluted with 1900 µl of hexane and then 1µl of each of the diluted essential oil samples were loaded into the auto sampler Gas Chromatograph- Mass Selector (GC-MS) system for analysis. The compounds of essential oil were eluted at different retention time and identified by the autosampler. Their percentage relative abundance was calculated using the formula (Abuto, 2016);

$$\% \text{ relative abundance} = \frac{\text{peak area of the compound}}{\text{Total sum of peak areas}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

The organic compounds were classified into different classes on the basis of presence of heteroatoms or the functional groups of the molecule.

3.6 Extraction of Crude Plant Extracts Using Different Solvents

3.6.1 Preparation of the Test Plant Materials

Fresh tickberry and Mexican marigold leaves were collected from the field while ginger rhizomes and garlic bulbs were bought from the local open air market and were all brought to the laboratory. For extraction of crude extracts, orne kilogram each of tickberry and Mexican marigold leaves were cleaned under tap water, rinsed in three changes of sterile distilled water and sterilized blotting paper was used to dry them. They were then separately dried in the oven at 40ºC for two days before being crushed to powder using a kitchen blender so as to increase the cell-solvent contact during extraction of the crude extracts. The dry powders were put in air tight dull bottles and stored for later use in the laboratory under room temperature. One kilogram of garlic cloves and ginger rhizomes were peeled separately, washed under tap water and rinsed in three changes of distilled water then dried using sterilized blotting papers. They were then chopped into small chunks using a sterilized scalpel and oven-dried at 40ºC for five days after which they were crushed to powder using a kitchen blender. The dry powders were also put in air tight dull bottles and stored for later use in the laboratory under room temperature.

3.6.2 Extraction of the Crude Plant Extracts

The extraction method followed a modified procedure illustrated by Handa *et al*. (2008). For water extraction, 200 grams of each powder (garlic, ginger, tickberry and Mexican marigold) was soaked separately in 500 ml of water for a period of 72 hrs. The content was then filtered using Whitman's (No.2) filter paper and formed stock solutions that were put in air tight bottles and stored in the refrigerator for later use in testing their efficacy on the test pathogens. Ethanol extraction involved 200 grams of each powder (garlic, ginger, tickberry and Mexican marigold) soaked separately in 500 ml of pure grade ethanol for a period of 72 hrs. The content was then filtered using Whitman's (No.2) filter paper and the solvent was evaporated in a vacuum evaporator to make concentrates that were stored in the refrigerator to be used to test their efficacy on the test pathogens. Methanol extraction involved 200 grams of each powder (garlic, ginger, tickberry and Mexican marigold) soaked separately in 500 ml of methanol for a period of 72 hrs. The content was then filtered using sterilized Whitman's (No.2) filter paper and a vacuum evaporator was used

to evaporate the solvent so as to make concentrates that were stored in the refrigerator to be used to test their efficacy on the fungal pathogens

3.7 *In-vitro* Efficacy of Crude Extracts and Essential oils on the Test Pathogens

3.7.1 *In-vitro* Efficacy of Crude Extracts on the Test Pathogens

The experiment aimed at testing *in-vitro* anti-microbial efficacy of the selected plant extracts on early and late blight pathogen of tomato and determining the most suitable extraction solvent to use in extracting crude plant extracts for control of early and late blight pathogens. The experiment was conducted at the University of Embu Microbiology Laboratory. Screening of the crude extracts to determine their inhibitory effects on pathogens causing early and late blight was done following modified technique of Al-samarrai *et al*., (2012; 2013). The *in-vitro* experiment was laid out in a completely randomized design (CRD), replicated four times.

Methanol and ethanol extracts were first diluted with 2ml of dimethyl sulfoxide (DMSO) before diluting with sterile distilled water in the ratio of 1:1. Five hundred (500) millilitres of Potato Dextrose Agar (PDA) and V8 agar was amended separately with 5ml of each of the four different extracts and dispensed into petri dishes. Five (5) ml of distilled water was mixed with the pathogen growth media for negative controls. One and half (1.5) grams of Ridomil Gold® (Metalaxyl-M and S-isomer, Mancozeb) was dissolved in 5ml of distilled water and mixed with the media as positive control. Five (5) millimetres culture discs from one week old cultures of *Alternaria solani* and *Phytophthora infestans* was cut using a sterilized cork borer and cultured at the centre of each petri-dish per replicate and incubated at room temperature (25^{o}C ±2). A pair of dividers and a ruler were used to measure the radial growth of each treatment after the 1st, 3rd, 5th and 7th day. The mean radial growth of the test pathogen on the amended media was compared with the negative and positive controls.

3.8.1 *In-vitro* Efficacy of Essential Oils on the Test Pathogens

In-vitro anti-microbial efficacy of four different essential oils from the selected plant extracts on early and late blight of tomato was tested. This was implemented following modified technique of Al-samarrai *et al*. (2012; 2013). The experimental design was a

completely randomized design (CRD), replicated four times. Five hundred (500) militres of PDA and V8 agar was amended separately with 1ml of each of the four different essential oils and dispensed into three petri dishes replicated four times. One (1) millilitre of distilled water was mixed with the media for the negative controls. One and half (1.5) grams of Ridomil Gold® synthetic fungicide (Metalaxyl-M and S-isomer, Mancozeb) was dissolved in 5ml of distilled water and mixed with the media as the positive controls. Five (5) millimetre culture discs from one week old cultures of *A. solani* and *P. infestans* were cut using a sterile cork borer and cultured at the centre of each petri-dish per replicate and incubated at room temperature. A pair of dividers and a ruler were used to measure radial growth of each treatment after the 1st, 3rd, 5th and 7th day. The mean radial growth of the test pathogen on the amended media was compared with the negative and positive controls.

3.8.2 *In-vivo* Efficacy of Essential Oils against Early and Late Blight of Tomato

In-vivo evaluation of the anti-microbial efficacy of the essential oils from the selected plants on late and early blight diseases of tomato was done with essential oils that were most effective in the *in-vitro* experiment (Garlic, Ginger and Mexican marigold). Fungicidal efficacy of the essential oils was also compared with commercial fungicide (Ridomil Gold® -Metalaxyl M and S-isomer, Mancozeb). Kilele F1 tomato seeds were bought from the local seed merchants and were sown in the screen house in plug trays with peat medium for easy transplanting. After three weeks, the seedlings were lifted from the plug trays and transplanted into individual plastic pots filled with sterilized potting mixture of sand, soil and well decomposed manure in the ratio of 3:2:1 in the green house.

Four plants were planted per row in plastic pots and arranged in a Randomized Complete Block Design (RCBD) and each treatment replicated four times. Pathogen isolates were sub-cultured for 14 days at 25ºC in an incubator before application. Spore suspension of *A. solani* and *P. infestans* was prepared by adding 5ml of sterile distilled water to a pure culture in a petri-dish. A bent glass rod was used to dislodge the spores and the content passed through a three-layer cheese cloth to remove the mycelia. Concentration of the spore suspension was determined using haemacytometer slide under a microscope and then adjusted to $1x10^6$ spores per millimeter with sterile water and the plants were sprayed with

the inoculums two weeks after transplanting. Symptoms of disease development began to appear on the 6th day.

Fourteen days after inoculation, plants were sprayed with different essential oils of Mexican marigold, garlic and ginger as foliar sprays per treatment. Ridomil Gold® (Metalaxyl M and S-isomer, Mancozeb) which is a curative fungicide was used as the positive control while plants sprayed with sterile water acted as the negative control. Five (5) mls of each essential oil was first mixed with 5 ml of tween 20 and then topped up to 500 mls of the solution using sterilized distilled water in preparation for spray. Ridomil® solution was prepared following the manufactures instructions where 1.5 g was mixed with 500 mls of water. Different 1L hand sprayers were used to spray individual plants per treatment. Tomato seedlings were sprayed with essential oils and ridomil synthetic fungicide per treatment two weeks after inoculation and then after every fourteen days up to 60 days after planting. Disease severity was recorded in each treatment after every 2 weeks using a scale of 0 – 5 proposed by Latha *et al*. (2009) as follows:

- 0 = healthy leaves with no visible lesions;
- 1= up to 10% of leaf area infected;
- 2= 11%-25% of leaf area infected;
- 3= 26-50% of leaf area infected;
- 4= 51-75% of leaf area infected; and
- 5= more than 75% of leaf area infected

Disease severity on each plant was determined by taking the average of disease scales per leaf. The disease scales were converted into percentage for each plant using the formula described by Chaerani *et al*. (2007) (Equation 1).

$$\text{Disease severity} = \frac{\text{Sum of all ratings} \quad X100}{(\text{No. of leaves sampled X maximum disease scale})} \quad \ldots\ldots\ldots\ldots 3.2$$

The efficacy of each treatment was calculated using the formula described by Derbalah *et al*. (2011) (Equation 2).

$$\% \text{ efficacy} = \frac{DSC-DST \ X \ 100}{DSC} \quad \ldots\ldots\ldots\ldots 3.3$$

Where: DSC = Disease severity under control; DST = Disease severity under treatment

Data was also collected on growth and yield parameters including number of branches, plant height, days to 50% flowering, number and weight of marketable fruits.

3.9 Data Analysis

Data on morphological characterization of *A. solani* and *P. infestans* was analysed through colony and microscopic examinations. Molecular identification was done by using Nucleotide Blast Program and sequence similarity searches done in the GenBank. Analysis of the chemical composition of essential oils was done using Autosampler Gas Chromatograph Mass Selector System. For the efficacy experiments the quantitative data obtained was subjected to Analysis of Variance (ANOVA) using XLSTAT version 2019 and separation of means was conducted using Student Newman's Keul (SNK) test at 95% level of confidence. Pearson correlation was conducted between growth and yield parameters and disease severity.

CHAPTER FOUR

RESULTS

4.1 Morphological Identification of the Pathogens

4.1.1 Identification of *Alternaria solani*

Twenty-four *Alternaria* isolates suspected to be *A. solani* varied in morphological characteristics in the growth medium (PDA). From the morphological observation the fungal isolates were grouped into five groups according to colony colour (front), substrate colour (reverse), growth pattern, growth margin and the morphology of the conidia. The colony colour varied between isolates (Table 4.1). Fungal isolates in group one had greenish brown colony colour and it was the largest comprising of twelve isolates. Group two had four isolates characterized by dark-brown colony colour. The fungal colony colour for group three which comprised of two isolates was dark grey while that of group four, made up of one isolate (A23) was grey.

Group five consisted of five isolates and was greyish-brown in colour. The substrate colour (reverse) also varied significantly between the isolate groups. For example, group one substrate colour was dark-brown, group two was dark-grey while group three was black (Plates 4.1- 4.5). The substrate in group four was brown with zonation while group five exhibited brownish-grey colour. The fungal growth pattern was irregular for group one while circular for all other groups. The colour on the fungal growth margins also varied between isolates. Group one had a major colour that was greyish-white while group two and five possessed growth margins that were brownish-white in colour.

The fungal growth margin for group three was greyish-white while group four was grey. Conidia characteristics also varied between isolate groups although they were all solitary. Conidia in group one and four had four transverse septa while groups two and three had conidia with three transverse septa. Conidia in group one and four lacked longitudinal septa while group two and three were muriform with one longitudinal septa each. Conidia in group five were muriform with one longitudinal septa and two transverse septa (Plates 4.1-4.5). The beaks of the conidia also varied significantly between the isolate groups. For example, groups one and three had elongated beaks but the beak for group three was

branched. Groups two and four had their conidia with short slender beaks while group five had short stout beaks (Table 4.1). The colour of the conidia was brown in all the groups while the mycelia for all the groups were septate, straight, hyaline and branched (Plate 4.6).

Table 4.1: Morphological variability of *A. solani* before DNA sequencing

Groups	**Isolates**	**Colony Colour**	**Substrate Colour**	**Margin Colour**	**Margin Growth**	**Transverse Septa**	**Longitudinal Septa**	**Beak Elongation**	**Beak Branching**
1	A1, A2, A8, A10, A12, A13, A14, A15, A16, A18, A20, A22	Greenish brown	Dark brown	Greyish white	Irregular	Four	None	Elongated	Unbranched
2	A4, A6, A9, A17	Dark brown	Dark grey	Brownish white	Circular	Three	One	Slender short	Unbranched
3	A5, A11	Dark grey	Black	White	Circular	Three	None	Elongated	Branched
4	A23	Grey	Brown with zonation	Grey	Circular	Four	None	Slender short	Unbranched
5	A3, A7, A19, A21, A24	Greyish brown	Greyish brown	Brownish white	Circular	Two	One	Stout short	Unbranched

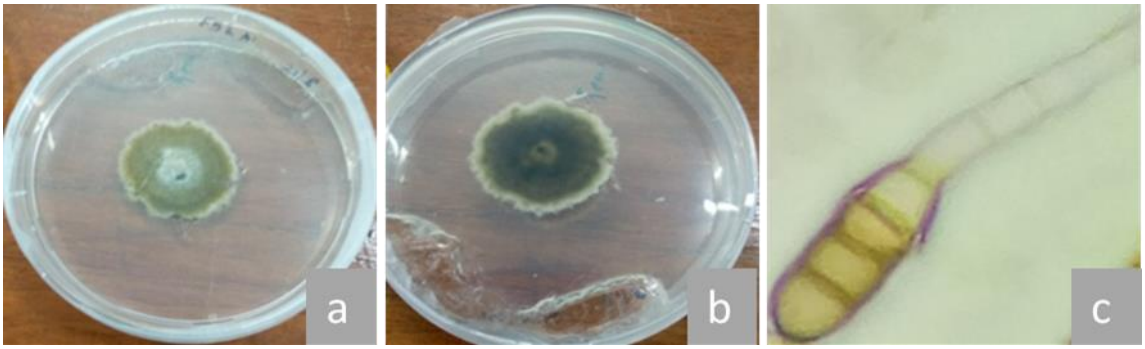

Plate 4.1: Group 1 of the *A. solani* isolates [Front (a), Reverse (b), Conidium (c)]

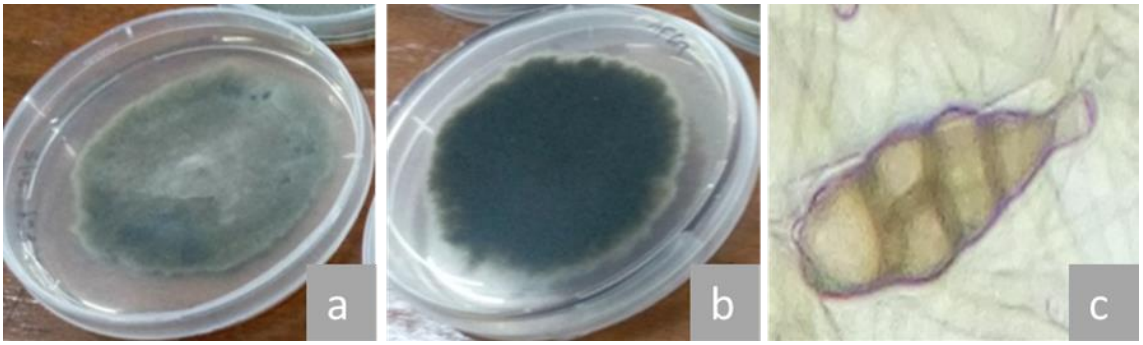

Plate 4.2: Group 2 of the *A. solani* isolates [Front (a), Reverse (b), Conidium (c)]

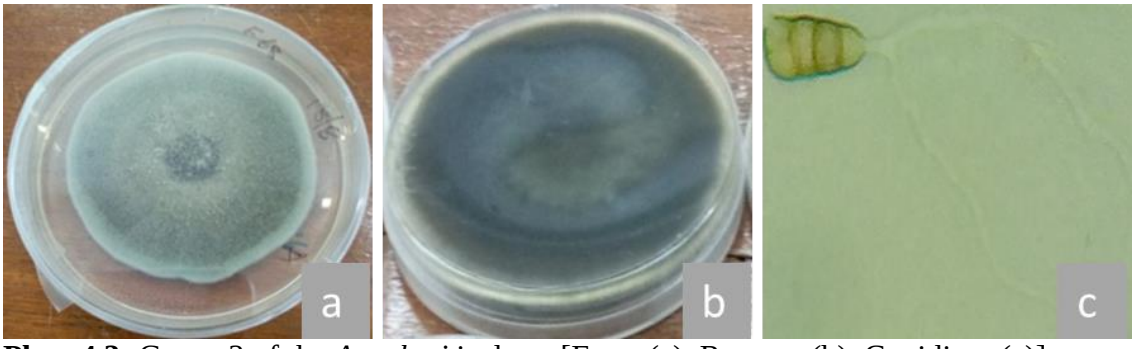

Plate 4.3: Group 3 of the *A. solani* isolates [Front (a), Reverse (b), Conidium (c)]

Plate 4.4: Group 4 of the *A. solani* isolates [Front (a), Reverse (b), Conidium (c)]

Plate 4.5: Group 5 of the *Alternaria* isolates [Front (a), Reverse (b), Conidia (c)]

Plate 4.6: Mycelia from the *Alternaria* fungal isolates

4.1.2 Identification of *Phytophthora infestans*

All the sixteen pathogen isolates suspected to be *P. infestans* did not vary in their morphological characteristics. The isolates had a white colony colour (front) and the substrate colour (reverse) was creamish. The growth pattern was circular with white margin colour (Plate 4.7 a, b). There were oospores with oogonia and amphigynous antheridia (Plate 4.7c). The mycelia were aseptate, multinucleate and heterothallic (Plate 4.7d).

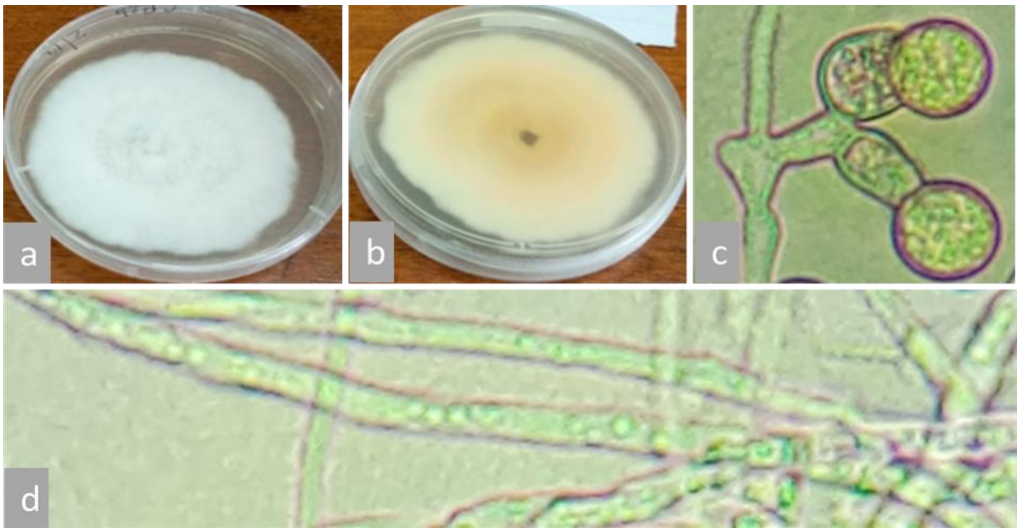

Plate 4.7: Morphology of *P. infestans* isolate - Front (a), Reverse (b), Sporangiophore (c), Mycelia (d)

4.1.3 Molecular Identification

4.1.3.1 Molecular Identification of *Alternaria solani*

The Internal Transcribed Sequences (ITS) amplification of the twenty-four (24) *A. solani* isolates by PCR resulted in a product of about 580 bp (Plate 4.8). The band size did not vary between the isolates. However, isolate number 24 did not amplify and thus could not be positively identified through sequencing.

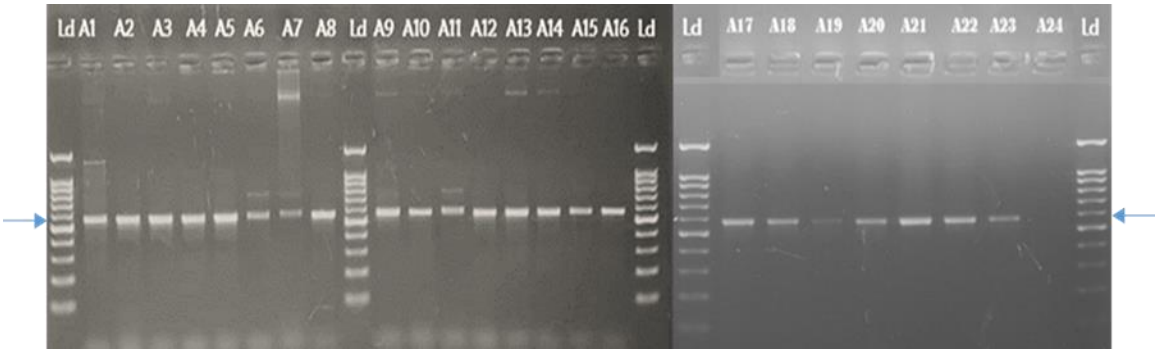

Plate 4.8: Gel image showing 580 bp (indicated by the arrow) of ITS1 and ITS4 regions of *Alternaria* spp, on 1.2% agarose gel electrophoresis. Ld is 100 bp ladder. A1-A24 are *A. solani* isolate numbers.

Sequence similarity searches of the 23 isolates whose ITS fragment amplified successfully was carried out using nucleotide BLAST program in the NCBI database. Nineteen (19) of them were positively identified as *A. solani* while the other four were identified as *A. alternata* (Table 4.2). Eleven *A. solani* isolates showed 100% nucleotide similarity with gene bank clone 105 that had GenBank accession number MN871613 together with isolate A1 which showed 99.42% nucleotide similarity with clone 105 in the gene bank. These had earlier been grouped under morphological group 1 and was the most predominant (52.18%) group among the pathogens isolated (Table 4.2).

Four other *A. solani* isolates (A4, A6, A9, A17) which were members of the morphological group 2 were found to be 100% similar to the gene bank clone 185 with accession number MN871616 and it was the second most predominant group (17.39%). Isolates A5 and A11 which belonged to the morphological group 3 matched 100% with gene bank clone 43 which had GenBank accession number MN871610 (Table 4.2). Isolate A23 which was the only one in the morphological group 4 was found to have 99.81% nucleotide similarity

with *A. solani* accessions in the gene bank but its closest clone could not be identified. This isolate had GenBank accession number LN879928 (Table 4.2). The accession MN871613 was the most prevalent with a total percent frequency of 52.18% followed by MN871616 (17.39%) and MN871610 (8.68%). The four isolates (A3, A7, A19, A21) that were identified to belong to *Alternaria alternata* had GenBank accession numbers MN822496, MN822565, KY570321 and MW009021 respectively and they showed 100% perfect match with different gene bank strains as shown in Table 4.2. The four belonged to morphological group 5 together with isolate 24 whose ITS fragment was not amplified.

Table 4.2: Molecular variability of *A. solani* and the related species after sequencing

Group	Isolates	Species Identity	Accession Number	Closest Match	Similarity (%) to GenBank accessions	Percent Frequency
1	A1	*A. solani*	MN871613	Clone 105	99.42	4.35
	A2, A8, A10 A12, A13, A14, A15, A16, A18, A20, A22	*A. solani*	MN871613	Clone 105	100	47.83
2	A4, A6, A9, A17	*A. solani*	MN871616	Clone 185	100	17.39
3	A5, A11	*A. solani*	MN871610	Clone 43	100	8.68
4	A23	*A. solani*	LN879928	Unidentified	99.81	4.35
5	A3	*A. alternata*	MN822496	BJ-YZ-14	100	4.35
	A7	*A. alternata*	MN822565	BJ-SB-41	100	4.35
	A19	*A. alternata*	KY570321	Strain Te 19	100	4.35
	A21	*A. alternata*	MW009021	DT1884-B	100	4.35

4.1.3.2 Molecular Identification of *Phytophthora infestans*

The ITS sequence amplification of the sixteen (16) *P. infestans* isolates by PCR resulted in a product of about 580 bp and there was no variation (Plate 4.9).

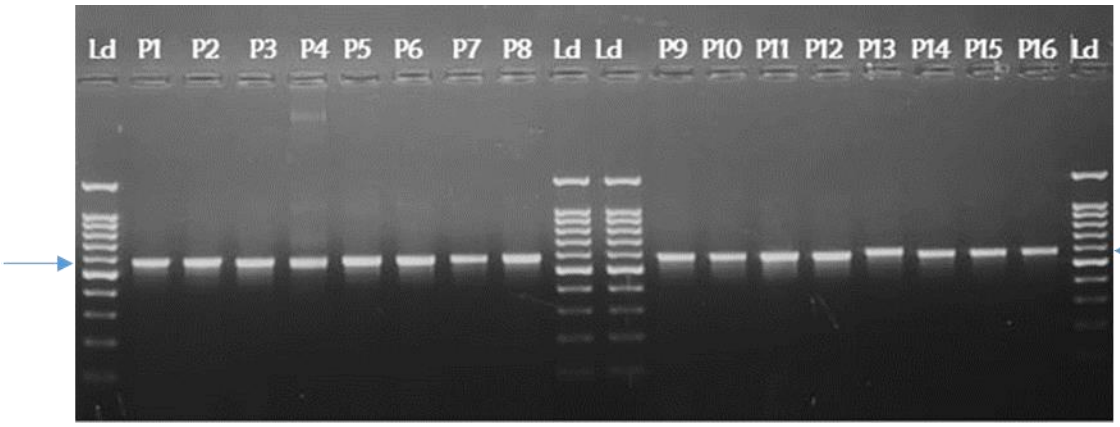

Plate 4.9: Gel image showing 580 bp (indicated the the arrow) of ITS1 and ITS4 regions of *P. infestans* spp on 1.2% agarose gel electrophoresis. Ld is 100 bp ladder. P1-P16 are the isolates numbers of *P. infestans*

Sequence similarity searches of the 16 isolates suspected to be *P. infestans* was carried out using nucleotide BLAST program which matched the isolates with GenBank accessions. The results showed that eleven of these isolates (68.75%) were positively identified as *P. infestans* with 100% genotypic similarity to Strain A2. They had accession number JX666330 from the GenBank. Three other isolates (P8, P15, P16) belonged to unspecified species of the genus Phytophthora and matched 100% with Strain Phy-1i. The three were in the GenBank accession number MT075724. The other two isolates (P6, P11) were identified as *Fusarium equiseti* with 100% resemblance to Strain P3B. The two had GenBank accession number MK571264.

Table 4.3: Molecular variability of isolates of *P. infestans* and related species after sequencing

Group	Isolates	Species Identity	Accession Number	Closest Match	Similarity (%) to GenBank accessions	Percent Frequency
1	P1, P2, P3, P4, P5, P7, P9, P10, P12, P13, P14	*P. infestans*	JX666330	Strain A2	100	68.75
2	P8, P15, P16	*Phytophthora sp.*	MT075724	Strain Phy-1i	100	18.75
3	P6, P11	*Fusarium equiseti*	MK571264	Strain P3B	100	12.5

4.1.3.3 Pathogenicity Test

Results of the pathogenicity test showed that the pathogens isolated from the inoculated tomato plants were the same as those that had been inoculated and the symptoms that developed from the inoculated plants were similar to those in the leaves collected from the tomato fields in Mwea. Plants inoculated with *P. infestans* developed pale green lesions on the leaves (Plate 4.10a) while those inoculated with *A. solani* developed brown lesions (Plate 4.10b). The conidia and the mycelium of *A. solani* were septate (Plate 4.11a) while *P. infestans* had aseptate mycelium and lemon-shaped sporangia arranged in simple and sympodia sporangiophore (Plate 4.11b).

Plate 4.10: Tomato plants infected by *P. infestans* (a) and *A. solani* (b)

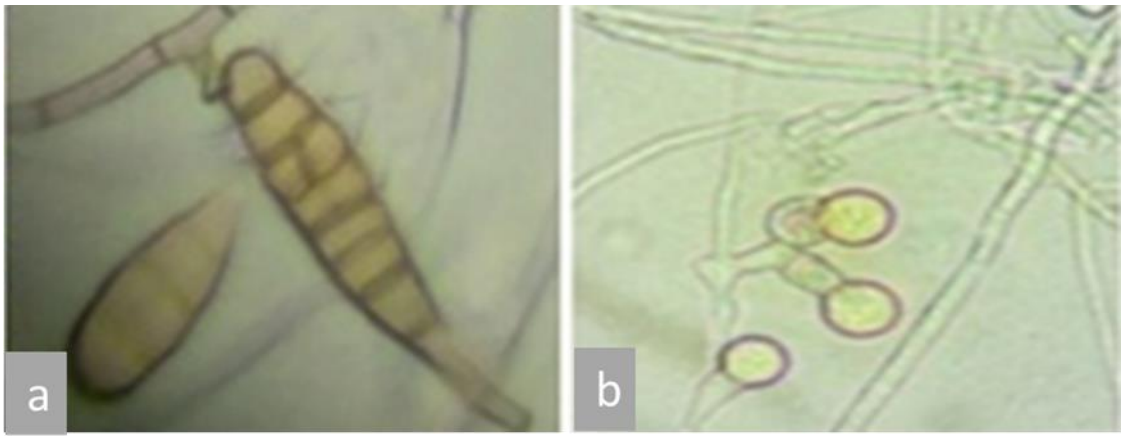

Plate 4.11: Conidia (a) of *A. solani* and sporangia (b) of *P. infestans* re-isolated from the inoculated tomato plants

4.2 Chemical Composition of the Essential Oils of the Test Plants

4.2.1 Classes of Compounds Identified from Essential Oils

A total of 52 different chemical classes were identified from the essential oils of the four different plants that were analysed during the study. Their percentage composition varied between the test plants (Table 4.4). Essential oils of Mexican marigold constituted the highest composition of the identified chemical classes at 71.2%, followed by ginger at 55.8%, while both tickberry and garlic oils constituted 53.8% of the total classes identified. There were 18 major classes that were identified with average percent chemical composition of >1% as shown in Table 4.4. Terpenes constituted the highest composition in the essential oils of all the four test plants. Other major chemical classes included organosulfurs, esters, steroids, alkanes, ketones, cycloalkane, aromatic hydrocarbons, alkanols, alkenols, carbaldehydes, cycloalkanols, aldehydes, organic acids, phthalate, alkenes, furans and carbonates listed in order of abundance (Table 4.4).

The composition of the different chemical classes in the essential oils of the tested plants was found to vary among the test plants. Ginger essential oil had the highest quantity of terpenes (42.18%) followed by garlic (39.57%), tickberry (37.26%) and Mexican marigold (20.94%). Mexican marigold had the highest amount of esters (14.01%) and relatively high amount of ketones (8.93%) and alkanols (4.73%). Ginger had the highest composition of ketones (8.99%) and fatty acids (3.67%) and relatively high amount of alkanes (7.42%) and cycloalkanols (4.13%). Garlic recorded the highest composition of organosulfur (15.01%), aromatic hydrocarbon (6.36%), carbonates (3.71%) and carbaldehyde (3.85%). Garlic also had relatively high amount of fatty acids (3.61%). Tickberry had the highest composition of alkanes (7.55%), cycloalkane (7.31), steroid (7.08%), alkanols (5.12%), alkenols (5.73%) and aldehydes (4.67%) as shown in Table 4.4. The composition of other minor compounds with average composition of less than 1% also varied among the test plants and were found to be present in either 3, 2 or 1 out of the 4 plants that were analysed except organic acid which was present in all the plants.

Table 4.4: Composition of chemical classes identified from different essential oils

S/NO.	Chemical Class	Percent Composition				Average
		Tick berry	Mexican marigold	Ginger	Garlic	
1.	Terpenes	37.26	20.94	42.18	39.57	34.99
2.	Ester	5.02	14.01	5.8	4.58	7.35
3.	Ketones	2.07	8.93	8.99	4.61	6.15
4.	Organosulfur	5.19	1.79	0.04	15.01	5.51
5.	Alkanes	7.55	0.87	7.42	3.20	4.76
6.	Cycloalkane	7.31	5.82	4.99	0.12	4.56
7.	Steroid	7.08	2.01	2.26	4.82	4.04
8.	Aromatic Hydrocarbon	0.73	4.16	4.37	6.36	3.91
9.	Alkanols	5.12	4.73	2.51	0.62	3.25
10.	Cycloalkanol	4.04	1.55	4.13		2.43
11.	Alkenols	5.73	0.54	2.73	0.59	2.40
12.	Carbonates		2.12	1.61	3.71	1.86
13.	Fatty acids			3.67	3.61	1.82
14.	Carbaldehyde	0.59	2.41	0.28	3.85	1.78
15.	Aldehydes	4.67	0.67	1.3		1.66
16.	Alkenes	0.12	5.32	0.48		1.48
17.	Ethers	0.07	2.56		2.53	1.29
18.	Carboxylic acid		2.81	1.12	0.32	1.06
19.	Alkaloids	0.09	3.50		0.06	0.91
20.	Organic Acid	0.21	2.59	0.24	0.21	0.81
21.	Heterocyclic hydrocarbon	0.03	0.43		2.49	0.74
22.	Hydroquinones		2.95			0.74
23.	Cyclic carboxylic ester(Lactone)		2.25			0.56
24.	Nitrile		0.15		1.81	0.49
25.	Furans	1.28	0.09	0.47		0.46
26.	Benzyl carbazate	1.67	0.09			0.44
27.	Vitamin	0.77	0.93		0.05	0.44
28.	Dione	1.71	0.02			0.43
29.	Amide	0.93		0.5	0.16	0.40
30.	Cycloketone(Cyclohexanone)			1.14		0.29
31.	Antihistamine		1.12			0.28
32.	Thiozole			1.05		0.26
33.	Polyphenol			1.04		0.26
34.	Hydroxysteroid				0.78	0.20
35.	Phthalate	0.11	0.06	0.51	0.04	0.18
36.	Silane		0.60		0.07	0.17
37.	Aromatic hydrocarbon(Toluene)		0.64			0.16
38.	Amine	0.03	0.39		0.08	0.13
39.	Chloroalkyne		0.45			0.11
40.	Cycloalkenol			0.07	0.26	0.08
41.	Thiophene	0.33				0.08
42.	Aromatic amine(Aniline)			0.29		0.07
43.	Isoprenoids	0.04			0.23	0.07
44.	Phenanthrenol		0.24			0.06
45.	Allethrin			0.21		0.05
46.	Phenol	0.17				0.04
47.	Corticosteroid		0.15			0.04
48.	Polycylic aromatic hydrocarbon				0.12	0.03
49.	Dye		0.07			0.02

50.	Bithiophene		0.07			0.02
51.	Pyrrolidinophenone			0.04		0.01
52.	Morpholine			0.04		0.01
	Total Compounds Found	**28 (53.8%)**	**37 (71.2%)**	**29 (55.8%)**	**28 (53.8%)**	

Further analysis of the composition of the chemical classes showed terpene compounds varied between the essential oil of the test plants. Table 4.5 shows the composition of some selected major chemical classes that were identified from the four test plants and the highest compound of the selected chemical classes in each of the test plants. The most abundant terpenes included bicyclogermacrene which is a sesquiterpene that was present in all the plants but highest in tickberry (5.24%) and ginger (3.49%). Others were monoterpenes such as 3- Carene and citral which were also present in all the essential oils of the test plants but most abundant in berry (Table 4.5). The major terpene in Mexican marigold was Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1S)- which was also present in all the other tested plants. In ginger, zingiberene was the most abundant terpene which was also present in garlic though in relatively smaller quantity. In garlic, 2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl) prop-2-en-1-ol was the most abundant terpene compound followed by Nerolidol. The former was also present in ginger and Mexican marigold while the later was also found in ginger (Table 4.5).

The only ester that was found in all the test plants was γ-Hexalactone (Table 4.5). In garlic, the highest ester compound was 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane (2.05%). Heptyltiglate, 4- was the most abundant (2.23%) ester compound in ginger. The most abundant esters in Mexican marigold were Chrysantenyl 2-methuylbutanoate (3.07%), Dodecanoic acid, 1,2,3-propanetriyl ester (2.85%) and 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a, 1b,4,4a,5,7a,8,9-octahydro-3-(h… (2.46%). In tick berry, Valeric acid, tridec-2-ynyl ester recorded the highest content among the ester compounds (Table 4.5). The most abundant ketone was Corymbolone which was found in the essential oils of all plants except Mexican marigold and was highest in ginger (3.59%). For example, Dihydrotagetone was only present in Mexican marigold essential oil. In garlic, the most abundant ketone was 6-Hepten-3-one,5-hydroxy-4-methyl- (1.57%) while in Mexican marigold, 1-Methoxybicyclo[2,2,2]oct-5-

en-2-yl methyl ketone recorded the highest content of 1.91%. In tick berry, 2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-… was the most abundant ketone (Table 4.5). Organosulfurs were majorly found in garlic with the leading being Allyl(Z)-prop-1-enyl trisulfide (4.30%) followed by (E)-1-Methyl-2-(prop-1-en-1-yl) disulfane (3.40%) and Diallylsulphide (2.94%).

Table 4.5: Most abundant compounds in selected major biochemical classes

S/No.	Chemical Class	Individual Compound	Tick berry	Mexican marigold	Ginger	Garlic
1.	Terpene	Bicyclogermacrene	5.24	0.11	3.49	0.57
2.	Terpene	3-Carene	4.36	0.39	2.89	0.08
3.	Terpene	Citral	2.72	1.39	0.47	0.11
4.	Terpene	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1S)-	0.15	2.66	0.14	1.88
5.	Terpene	Zingiberene			3.55	0.32
6.	Terpene	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol		0.06	2.29	3.93
7.	Terpene	Nerolidol			0.12	3.63
8.	Ester	γ-Hexalactone	0.05	0.04	0.05	0.05
9.	Ester	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane				2.05
10.	Ester	Heptyltiglate, 4-			2.23	
11.	Ester	Chrysantenyl 2-methuylbutanoate		3.07		
12.	Ester	Dodecanoic acid, 1,2,3-propanetriyl ester		2.85		
13.	Ester	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a, 1b,4,4a,5,7a,8,9-octahydro-3-(h…		2.46		
14.	Ester	Valeric acid, tridec-2-ynyl ester	2.69			
15.	Ketone	6-Hepten-3-one,5-hydroxy-4-methyl-				1.57
16.	Ketone	Corymbolone	0.53		3.59	0.86
17.	Ketone	1-Methoxybicyclo[2,2,2]oct-5-en-2-yl methyl ketone		1.92		
18.	Ketone	2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-…	1.28			
19.	Organosulfur	Allyl(Z)-prop-1-enyl trisulfide				4.30
20.	Organosulfur	(E)-1-Methyl-2-(prop-1-en-1-yl) disulfane				3.40
21.	Organosulfur	Diallyl sulfide				2.94

The study further identified most common compounds that were present in the essential oils of all the test plants as shown in Table 4.6. Majority were terpenes (5), followed by alkanes (2), while carbaldehyde, phthalate and ester had one compound each that was

common in all the four plants. In addition, 16 compounds that were conspicuously missing in only one out of the four tested plants were identified (Table 4.6). Ginger was the only plant that did not contain cholesterol.

Table 4.6: Compounds with broad presence in the test plants

S/No.	Chemical Class	Individual Compound	Tick berry	Mexican marigold	Ginger	Garlic
1.	Terpene	Bicyclogermacrene	5.24	0.11	3.49	0.57
2.	Terpene	3-Carene	4.36	0.39	2.89	0.08
3.	Carbaldehyde	Longifolenaldehyde	0.59	0.93	0.21	3.86
4.	Terpene	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	0.15	2.66	0.14	1.88
5.	Terpene	Citral	2.72	1.39	0.47	0.11
6.	Terpene	Caryophyllene	0.16	0.05	0.64	0.35
7.	Phthalate	Bis(2-ethylhexyl)phthalate	0.12	0.06	0.56	0.05
8.	Alkane	Hexane,3-methyl-4-methylene-	0.05	0.35	0.04	0.06
9.	Alkane	Dodecane, 1-fluoro-	0.17	0.23	0.02	0.04
10.	Ester	γ-Hexalactone;	0.05	0.04	0.05	0.05
11.	Steroid	Cholesterol	4.71	1.97		3.43
12.	Terpene	Sabinene	0.06	0.15	1.81	
13.	Terpene	Linalool	0.05	0.17	0.32	
14.	Organosulfur	2,2-Dimethyl-propyl2,2-dimethyl-propanesulfinyl sulfone	0.14	0.05	0.04	
15.	Cycloalkane	Nonane, 2,2,4,4,6,8,8-heptamethyl-	0.51	0.04	0.62	
16.	Aromatic Hydrocarbon	m-Xylene; m-Xylol;	0.73	0.06	0.65	
17.	Terpene	L-.alpha.-Terpineol	0.07		0.32	0.05
18.	Terpene	.beta.-Guaiene	0.14		2.15	1.16
19.	Terpene	.alfa.-Copaene	0.25		2.26	0.46
20.	Amide	Piperidene	0.93		0.08	0.09
21.	Alkenol	(1R,7S,E)-7-Isopropyl-4, 10-dimethylenecyclodec-5-enol	3.96		2.74	0.60
22.	Alkane	4,4-Dimethyl octane	3.34		3.21	0.04
23.	Terpene	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol		0.06	2.29	3.93
24.	Terpene	Borneol		0.10	0.04	2.13
25.	Carboxylic acid	Hematoporphyrin		2.81	1.18	0.32

The study also identified twenty rare compounds that only occurred in one out of the four plants that were tested. Most of these compounds were found in Mexican marigold which had 9 compounds which were not present in the other tested plants. This was followed by ginger with 7 unique compounds. Both tickberry and garlic had 2 compounds each that were not present in the other test plants (Table 4.7).

Table 4.7: Compounds that were found to be rare in the test plants

S/NO.	Test plant	Chemical Class	Actual Compound
1.	Mexican marigold	Hydroquinones	tert-Butylhydroquinone
2.	Mexican marigold	Cyclic carboxylic ester (Lactone)	.beta.-Cyclocostunolide
3.	Mexican marigold	Antihistamine	Ethylamine,2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl-
4.	Mexican marigold	Aromatic hydrocarbon (Toluene)	p-Ethyltoluene (4- Ethyltoluene)
5.	Mexican marigold	Chloroalkyne	7-Heptadecyne, 1-chloro-
6.	Mexican marigold	Phenanthrenol	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro…
7.	Mexican marigold	Corticosteroid	(3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione
8.	Mexican marigold	Dye	4,5,6,7-Tetrachlorofluorescein
9.	Mexican marigold	Bithiophene	5-(But-3-ene-1-ynyl)-2,2'-bithienyl
10.	Ginger	Cycloketone(Cyclohexanone)	Cryptone
11.	Ginger	Thiozole	Thiozole
12.	Ginger	Polyphenol	Cubebanol
13.	Ginger	Aromatic amine(Aniline)	Octanal, (2,4-dinitrophenyl)hydrazone
14.	Ginger	Allethrin	Bioallethrin
15.	Ginger	Pyrrolidinophenone	2-Methyl-a-Pyrrolidinopropiophenone
16.	Ginger	Morpholine	4-(Pyrrolidin-2-ylmethyl)morpholine
17.	Tick berry	Thiophene	(1R,2S,6S,7S,8S)-8-Isopropyl -1-methyl -3-methylenetricyclo[4.4.0.02,7]decan…
18.	Tick berry	Phenol	Diepicedrene-1-oxide
19.	Garlic	Hydroxysteroid	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trih…
20.	Garlic	Polycyclic aromatic hydrocarbon	3,6-Dimethoxy- 1a,2,2a,3,6,6a,7,7a-octahydro-1-oxacyclopropa[b]naphthalene

4.3 Effects of the Crude Extracts on the Test Pathogens

4.3.1 Effect of Water Extracted Crude Extracts on the Test Pathogens

The results obtained from the current study indicated that all the plant extracts were effective in inhibiting the growth of the test pathogens as compared to the negative control (Table 4.8). However, the rate of inhibition varied significantly ($p<0.0001$) between the test plant extracts as compared to the positive and negative controls within a period of seven days. Both the test pathogens showed increased growth from day one to day seven in all the treatments except for the positive control (Ridomil synthetic fungicide®) where

there was 100% pathogen growth inhibition. On the first day, growth of *A. solani* on the negative control and the Mexican marigold treatment did not vary significantly. The growth of the same pathogen on tick berry and ginger extracts did not vary significantly.

Growth of the pathogen on ginger and garlic treatments varied significantly ($p<0.0001$) on the first day. Garlic was the most effective as it had the highest rate of pathogen growth inhibition among the extracts on *A. solani* on the first day. On the third day, growth of *A. solani* varied significantly ($p<0.0001$) between treatments. However, the test pathogens exhibited a similar growth pattern between tickberry, ginger and garlic treatments. The negative control had the highest pathogen growth. On the fifth and the seventh day, growth of *A. solani* varied significantly ($p<0.0001$) between the treatments. The pathogen grew more on the negative control than the test plant extracts. Among the plant crude extracts, growth of the pathogen was more on Mexican marigold and less on garlic treatment.

P. infestans had a similar growth on Mexican marigold, tickberry and ginger treatments on the first day. However, the growth varied significantly ($p<0.0001$) between the treatments and the controls (Table 4.8). Garlic treatment seemed to have a higher antimicrobial activity against the test pathogen as compared to ginger, tickberry and Mexican marigold treatments. Ridomil synthetic fungicide had 100% pathogen growth inhibition throughout the experimental period. On the third day, *Phytophthora* growth also varied significantly ($p<0.0001$) between the treatments and the controls. Growth of the pathogen on Mexican marigold and tickberry treatments did not vary significantly. Among the plant extracts, garlic had the highest antifungal activity followed by ginger. The fifth and the seventh day also followed a similar trend and growth of *Phytophthora* varied significantly ($p<0.0001$) between the treatments. Among the plant extracts the antimicrobial activity was higher in garlic, followed by ginger, tickberry and the least fungicidal activity was in Mexican marigold extract.

Table 4.8: Efficacy of water extracted plant extracts on pathogen growth

Test Material	Growth of Pathogen Isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	3.59^{a}	20.08^{a}	41.92^{a}	61.67^{a}	6.00^{a}	27.25^{a}	45.25^{a}	64.42^{a}
Marigold	3.67^{a}	19.00^{b}	37.25^{b}	56.17^{b}	3.42^{b}	18.59^{b}	37.67^{b}	56.17^{b}
Tick berry	2.92^{ab}	17.00^{c}	34.75^{c}	51.00^{c}	4.00^{b}	18.34^{b}	36.33^{c}	54.50^{c}
Ginger	2.42^{b}	16.25^{c}	33.42^{d}	47.75^{d}	3.25^{b}	16.75^{c}	31.92^{d}	49.83^{d}
Garlic	1.75^{c}	16.84^{c}	31.59^{e}	45.17^{e}	2.17^{c}	14.75^{d}	28.25^{e}	37.67^{e}
Ridomil	0.00^{d}	0.00^{d}	0.00^{f}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.212	0.255	0.400	0.414	0.335	0.358	0.315	0.433
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at p=0.05. SE is the standard error; DF is the degrees of freedom

4.3.2 Effect of Methanol Extracted Crude Extracts on the Test Pathogens

From the results of the study, it was noted that all the methanol extracted plant extracts had biocontrol activity against the test pathogens but their antimicrobial activity varied significantly ($p<0.0001$) as compared to the negative and positive controls (Table 4.9). The growth of *A. solani* on the first day seemed to vary significantly ($p<0.0001$) between the treatments. Mexican marigold and tickberry extracts had similar effect on pathogen growth but recorded pathogen growth inhibition as compared to the negative control. Ginger and garlic also had a similar effect on pathogen growth better than Mexican marigold and tickberry extracts but lesser than the positive control. Ridomil® synthetic fungicide had 100% pathogen growth inhibition throughout the testing period.

The third day and the seventh day had similar pattern, where pathogen growth varied significantly ($p<0.0001$) between treatments. The negative control had the highest pathogen growth. Among the plant extracts, garlic had the highest antifungal activity followed by ginger while Mexican marigold had the lowest pathogen growth inhibition. On the fifth day, the growth of the pathogen also varied significantly where the highest growth of *Alternaria* was in the negative control. Among the plant extracts Mexican marigold and tickberry had similar effect on the pathogen growth while garlic had the highest antifungal activity followed by ginger. During the testing period, garlic extracts had the highest antimicrobial activity against *A. solani* followed by ginger, tickberry and Mexican marigold (Table 4.9).

The growth of *P. infestans* varied significantly ($p<0.0001$) between treatments on the first day. The negative control exhibited the highest pathogen growth while the positive control exhibited excellent antifungal activity of 100% growth inhibition during the entire experimental period. Among the plant extracts, garlic had the highest biocontrol on the pathogen followed by ginger and then Mexican marigold. Tickberry treatment had the lowest antifungal activity among the plant extracts. On the third day, *P. infestans* growth also varied significantly ($p<0.0001$) between treatments. Among the plant extracts, garlic had the highest antifungal activity against the *P. infestans*, followed by ginger while tickberry extract had the lowest antimicrobial activity. On the fifth day, pathogens' growth inhibition on tick berry and ginger did not vary significantly. The lowest biocontrol activity was noted in Mexican marigold extract. On the seventh day, *P. infestans* growth on the treatments varied significantly between the treatments. The highest pathogen growth was noted in the negative control. Among the tested plant extracts, the highest pathogen growth inhibition was observed in garlic, followed by tickberry, ginger and last was Mexican marigold (Table 4.9).

Table 4.9: Efficacy of methanol extracted plant extracts on pathogen growth

Test Material	Growth of Pathogen Isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	5.00[a]	27.25[a]	47.75[a]	64.92[a]	5.33[a]	31.58[a]	62.08[a]	76.75[a]
Marigold	3.67[b]	20.17[b]	36.42[b]	52.50[b]	3.83[c]	20.25[c]	38.67[b]	54.25[b]
Tick berry	3.08[b]	19.00[c]	36.09[b]	50.25[c]	4.75[b]	26.09[b]	37.25[c]	47.83[d]
Ginger	1.92[c]	12.42e	30.00[c]	42.67[d]	2.50[d]	18.09[d]	36.42[c]	50.33[c]
Garlic	2.17[c]	15.83[d]	28.00[d]	39.75[e]	1.50[e]	13.09[e]	24.67[d]	35.08[e]
Ridomil	0.00[d]	0.00[f]	0.00[e]	0.00[f]	0.00[f]	0.00[f]	0.00[e]	0.00[f]
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.262	0.297	0.575	0.286	0.192	0.618	0.449	0.270
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at p=0.05.
SE is the standard error; DF is the degrees of Freedom

4.3.3 Effect of Ethanol Extracted Crude Extracts on the Test Fungal Pathogens

All the ethanol extracted plant extracts and the positive control were effective in inhibiting the growth of the test pathogens (Table 4.10) but the efficacy in growth inhibition between test plants varied significantly ($p<0.0001$). *A. solani*, growth did not vary significantly on

the first day for ginger and Mexican marigold treatment. Ridomil synthetic fungicide treatment recorded 100% growth inhibition throughout the test period. Garlic extract recorded the highest antimicrobial activity among the plant extracts. On the third day, *A. solani* growth on Mexican marigold was higher than the negative control. Garlic treatment had the lowest pathogen growth followed by ginger. On the fifth day, growth of the test pathogen did not vary significantly between the Mexican marigold and the negative control, but between the other treatments, there was a significant pathogen growth variation. The highest antifungal activity was observed in the positive control, followed by garlic and then tickberry. On the seventh day, the pathogen growth varied significantly ($p<0.0001$) between treatments. The highest incremental pathogen growth was noted in the negative control. Among the plant extracts, the lowest antifungal activity was noted in Mexican marigold treatment while the highest biocontrol activity was in garlic treatment.

The efficacy of the test plant extracts varied significantly ($p<0.0001$) on *P. infestans.* On the first day, the pathogen growth in the negative control, Mexican marigold, tickberry and ginger did not vary significantly. Garlic extract had a similar effect with ridomil synthetic fungicide on the test pathogen growth. Garlic and Ridomil® synthetic fungicide were the most effective in inhibiting the growth of *P. infestans*. On the third and fifth day, growth of the test pathogen varied significantly between the treatments. Among the test plants, garlic had the highest antimicrobial activity against *P. infestans* followed by ginger, tickberry and Mexican marigold in that order. On the seventh day, the pathogen growth between Mexican marigold and tickberry did not vary significantly. Ridomil® synthetic fungicide treatment had no pathogen growth throughout the test period. Among the test plant extracts, garlic had the highest growth inhibition on the test pathogen followed by ginger, tickberry and lastly Mexican marigold.

Table 4.10: Efficacy of ethanol extracted plant extracts on pathogen growth

Test Material	Growth of Pathogen Isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	5.17^{a}	22.83^{b}	39.67^{a}	61.25^{a}	5.25^{b}	26.75^{a}	53.42^{a}	69.59^{a}
Marigold	5.00^{a}	24.50^{a}	40.67^{a}	51.25^{b}	4.84^{b}	22.25^{b}	40.83^{b}	50.00^{b}
Tick berry	2.67^{b}	13.50^{d}	28.50^{c}	47.58^{c}	2.67^{b}	14.67^{c}	32.33^{c}	49.34^{b}
Ginger	5.50^{a}	19.75^{c}	30.75^{b}	40.59^{d}	2.42^{b}	11.00^{d}	26.42^{d}	42.25^{c}
Garlic	1.42^{c}	9.42^{e}	22.83^{d}	34.50^{e}	0.00^{c}	5.00^{e}	17.59^{e}	29.75^{d}
Ridomil	0.00^{d}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{c}	0.00^{f}	0.00^{f}	0.00^{e}
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.203	0.300	0.428	0.404	0.169	0.287	0.568	0.344
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at p=0.05. SE is the standard error; DF is the degrees of Freedom

4.3.4 Effects of Extraction Solvents on Antimicrobial Efficacy of the Crude Extracts

The antimicrobial activity of the crude extracts on the test pathogens varied significantly ($p<0.05$) with the solvent used in extraction of the test plant extracts. Results showed that the ethanol extracted crude extracts had the best pathogen growth inhibition at the end of the testing period (Day 7). Methanol and water extracts followed and had the lowest pathogen growth inhibition (Fig. 4.1). On the other hand, growth of the two test pathogens did not vary significantly ($p>0.05$) within the same extraction solvent.

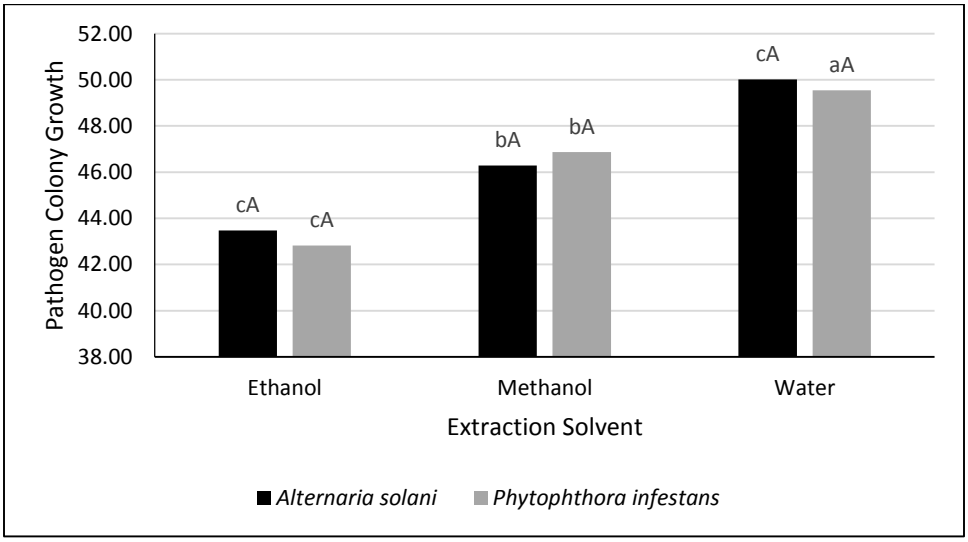

Figure 4.1: Effects of extraction solvent on efficacy of crude extracts on test pathogens. Means with the same lower case letter on different extraction solvents are not significantly different while those with the same upper case letter on the same extraction solvent are not significantly different at p = 0.05.

4.3.5 Comparative Response of the Two Test Pathogens to the Crude Extracts

The results obtained from the current study showed that growth of *A. solani* and *P. infestans* did not vary significantly in both tickberry and Mexican marigold extracts, but in ginger and garlic extracts, the test pathogens' growth varied significantly ($p<0.05$). However, *A. solani* growth was lower in ginger extracts than *P. infestans* while garlic extracts portrayed a higher antimicrobial activity against *P. infestans* than *A. solani* (Fig. 4.2).

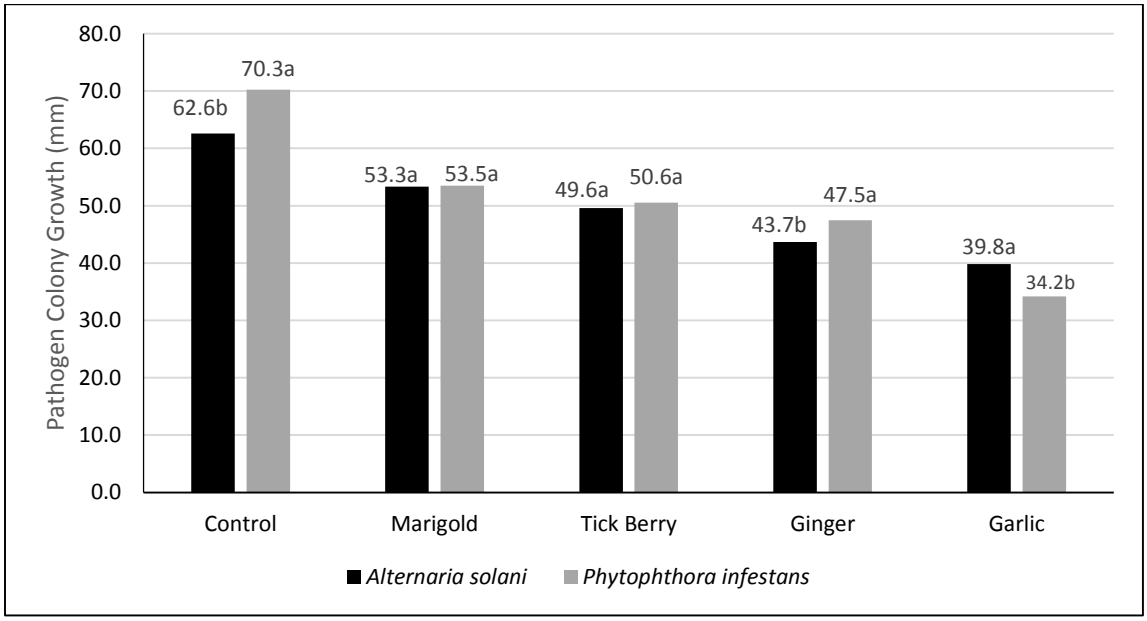

Figure 4.2: Comparative response of the two test pathogens to the crude extracts. Means with the same letter on the bars are not significantly different under the same treatment (test plant) at $p = 0.05$.

4.3.6 Antimicrobial Efficacy of the Essential Oils on the Test Pathogens

All the essential oils from the four test plants showed biocontrol activity against the test pathogens (Table 4.11) and their antimicrobial activity varied significantly ($p < 0.0001$). Ginger and garlic essential oils had excellent antimicrobial activity against all the test pathogens as Ridomil® synthetic fungicide treatment throughout the experimental period (Table 4.11). The antimicrobial activity of Mexican marigold and tickberry essential oils also varied significantly ($p < 0.0001$) on the growth of *A. solani* Mexican marigold essential oil was more effective than tickberry essential oil. On *P. infestans*, the results seemed a bit different than that of *A. solani*. On the first day the antifungal activity of Mexican marigold and tickberry essential oil on the growth inhibition of *P. infestans* varied significantly with tickberry essential oil being more effective than Mexican marigold. For the rest of the

experimental period, the antimicrobial activity of tickberry and Mexican marigold essential oils on *P. infestans* did not vary significantly but they were effective compared to the negative control (Table 4.11).

Table 4.11: Antimicrobial activity of essential oils on the growth of the test pathogens

Test Material	Growth of Pathogen Isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	4.67[a]	25.33[a]	43.42[a]	59.67[a]	4.75[a]	27.17[a]	50.75[a]	68.67[a]
Marigold	1.84[c]	7.17[c]	21.59[c]	31.67[c]	1.83[b]	8.92[b]	21.33[b]	28.58[b]
Tick berry	2.92[b]	10.25[b]	23.25[b]	35.25[b]	1.25[c]	8.83[b]	22.09[b]	29.00[b]
Ginger	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Garlic	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Ridomil	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.228	0.372	0.441	0.644	0.115	0.357	0.484	0.624
DF	5	5	5	5	5	5	5	5

Means followed by the same letter within the column are not significantly different at p=0.05; SE - Standard Error; DF - Degrees of Freedom

4.3.7 Comparative Response of Essential Oils and Crude Extracts to Test Pathogens

From the results of the current study, it was evident that all the test plants had antimicrobial potential against the growth of the test pathogens as compared to the negative and positive controls (Fig. 4.3). However, essential oils were more effective than the test plants crude extracts in inhibiting the growth of the test pathogens. Essential oils from ginger and garlic had 100% pathogen growth restriction similar to Ridomil® synthetic fungicide. Likewise, the efficacy of tickberry and Mexican marigold improved more when essential oil was used compared to crude extracts from the same plant.

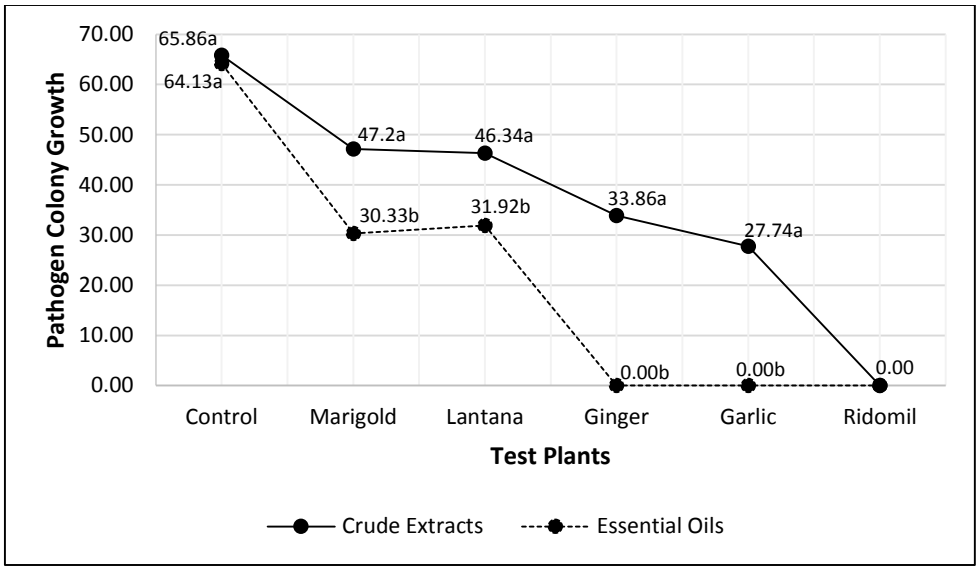

Figure 4.3: Comparative effects of crude extracts and essential oils on the test pathogens. Means with the same letter under the same test plant extract are not significantly different based on SNK test at p = 0.05

4.3.8 Effects of the Extraction Method of Plant Extracts on the Test Pathogens

The growth inhibition of the test pathogens was influenced significantly ($p<0.0001$) by the method used to extract the biocontrol products (Table 4.12). Essential oils were found to perform significantly better than solvent extracted crude extract. The solvent of extraction was also found to significantly influence the efficacy of the crude extracts on pathogen growth inhibition. On the first day, *A. solani* growth in water and methanol extracted extracts did not vary significantly. Ethanol extracted extract had the lowest growth inhibition of *A. solani* while essential oils had excellent growth inhibition throughout the testing period. On the third day, the efficacy of water and ethanol extracted extracts did not vary significantly on *Alternaria* growth inhibition. The lowest pathogen growth restriction was noted in methanol extracted extracts. On the fifth day, growth of *Alternaria* in water and methanol extracted extracts did not vary significantly. On the seventh day, growth of the test pathogen varied significantly between the extraction solvents and essential oils. The highest antimicrobial activity on *A. solani* was noted in ethanol extracted extract, followed by methanol extracted extracts and lastly water extracted extracts (Table 4.12).

The test pathogen growth also varied significantly between the extraction solvents on *P. infestans*. On the first day, methanol extracted extracts performed better than water and

ethanol extracted extracts which had similar effects on the test pathogen. Essential oils were the most effective throughout the test period. On the third and fifth day, the pathogen growth varied significantly ($p<0.0001$) between the extraction solvents. The lowest antifungal activity was noted in methanol extracted extracts while ethanol extracted extract had the best fungicidal activity. On the seventh day, water and methanol extracted extracts had similar effects. Extracts from ethanol had a higher pathogen growth inhibition as compared to extracts from water and methanol.

Table 4.12: Effects of the extraction methods on the growth of the test pathogens

Extraction Solvent	Growth of Pathogen Isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Water	2.39^{b}	14.86^{b}	29.82^{a}	43.62^{a}	3.14^{a}	15.94^{b}	29.90^{b}	43.76^{a}
Methanol	2.64^{b}	15.78^{a}	29.71^{a}	41.68^{b}	2.99^{a}	18.18^{a}	33.18^{a}	44.04^{a}
Ethanol	3.29^{a}	15.00^{b}	27.07^{b}	39.19^{c}	2.53^{b}	13.28^{c}	28.43^{c}	40.15^{b}
Essential Oils	1.57^{c}	7.12^{c}	14.71^{c}	21.10^{d}	1.31^{c}	7.49^{d}	15.69^{d}	21.04^{c}
Pr> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means followed by the same letter within column are not significantly different at p=0.05.

4.3.9 Interaction Between the Pathogens and Extraction Solvents and Test Plant Extracts

A combined analysis of variance showed that there was significant ($p<0.05$) interaction between the pathogens and the extraction method in all the test plant extracts throughout the testing period except on day 5 for Mexican marigold (Table 4.13). On the other hand, there was significant ($p<0.05$) interaction between the test pathogens and the test plant extracts in all the extraction methods throughout the testing period.

Table 4.13: Interaction between the pathogen and extraction solvent and the test plant

Test Material	*Pathogen x Test Material Interaction*				Extraction Solvent	*Pathogen x Extraction solvent Interaction*			
	Day 1	**Day 3**	**Day 5**	**Day 7**		**Day 1**	**Day 3**	**Day 5**	**Day 7**
Marigold	0.005	<0.0001	0.540^{NS}	0.003	Water	0.002	<0.0001	<0.0001	<0.0001
Tick berry	<0.0001	0.005	<0.0001	<0.0001	Methanol	<0.0001	<0.0001	<0.0001	<0.0001
Ginger	<0.0001	<0.0001	<0.0001	<0.0001	Ethanol	<0.0001	<0.0001	<0.0001	<0.0001
Garlic	0.0005	<0.0001	<0.0001	<0.0001	E. Oils	0.013	0.001	<0.0001	<0.0001
DF	3	3	3	3	DF	5	5	5	5

NS -Not Significant; DF -Degrees of Freedom; E. Oils - Essential Oils

4.4 In-vivo Efficacy of Essential Oils on Disease Severity, Growth and Yield of Tomato

4.4.1 Effects of the Essential Oils on the Leaf Number of Tomato

The number of leaves on the tomato plants inoculated with *A. solani* isolate varied between the treatments but the leaves increased in number as the plants continued to grow (Table 4.14). Among the tomato plants inoculated with *A. solani*, the number of leaves was not significantly different ($p > 0.05$) in all the treatments in the second week. In week four, tomato plants treated with Mexican marigold essential oil had the lowest number of leaves, but did not differ significantly with the control. In week six the number of leaves did not vary significantly ($p > 0.05$) between treatments. However, week eight had significantly ($p < 0.05$) lower number of leaves in the control but did not differ significantly in the other treatments. In week ten, the number of leaves varied significantly ($p < 0.05$) between control and the treatments. The number of leaves on tomato plants treated with ginger, garlic and Mexican marigold essential oil did not vary significantly but they were significantly higher than in plants treated with ridomil® synthetic fungicide and the control.

In tomato plants inoculated with *P. infestans*, the number of leaves increased in all the treatments as the plants continued to grow (Table 4.14). There was no significant ($p > 0.05$) difference in leaf number between all treatments in week two and four. In week six and eight, there was significant ($p<0.05$) difference in leaf number between treatments. Mexican marigold essential oils maintained the highest number of leaves while the control treatment had the lowest number. In week ten, only Mexican marigold treatment differed significantly from the control while ginger, garlic and ridomil® synthetic fungicide did not differ significantly from the control treatment. The plants treated with Mexican marigold essential oils also appeared to have more vigour as compared visually with other treatments.

Table 4.14: Effects of essential oil on leaf number of inoculated tomato plants

	Number of leaves on inoculated tomato plants									
	Alternaria solani					***Phytophthora infestans***				
Test Material	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**
Control	9.00^{a}	9.75^{ab}	11.75^{a}	11.75^{b}	12.75^{c}	6.25^{a}	8.00^{a}	11.25^{c}	12.00^{c}	13.75^{b}
Marigold	8.50^{a}	9.00^{b}	11.50^{a}	14.00^{ab}	18.50^{a}	6.25^{a}	8.75^{a}	19.50^{a}	20.00^{a}	20.50^{a}
Garlic	9.25^{a}	10.00^{ab}	13.75^{a}	14.25^{ab}	19.50^{a}	6.25^{a}	8.00^{a}	16.75^{b}	17.50^{b}	17.50^{ab}
Ginger	8.25^{a}	10.25^{ab}	13.50^{a}	15.25^{a}	18.50^{a}	6.25^{a}	8.00^{a}	15.00^{b}	16.00^{b}	15.75^{b}
Ridomil	8.50^{a}	11.25^{a}	14.40^{a}	15.75^{a}	15.00^{b}	6.25^{a}	8.75^{a}	14.00^{b}	15.50^{b}	17.50^{ab}
Pr>F	0.112^{NS}	0.049	0.060^{NS}	0.050	<0.0001	0.943^{NS}	0.166^{NS}	0.001	<0.0001	0.013
SE	0.431	0.459	0.771	0.704	0.447	0.258	0.320	0.840	0.592	0.928
DF	4	4	4	4	4	4	4	4	4	4

Means followed by the same letter within a column are not significantly different at p=0.05; Wk -Week; SE -Standard Error; DF -Degrees of Freedom; NS – Not Significant

4.4.2 Effect of the Test Pathogens on Leaf Number of Tomato

The number of leaves increased in all the tomato plants inoculated with the test pathogens as the weeks progressed (Table 4.15). However, the number of leaves varied significantly ($p < 0.0001$) between tomato plants inoculated with different test pathogens in the first eight weeks (Table 4.15). More leaves were recorded in tomato plants inoculated with *A. solani* as compared to *P. infestans*. The number of tomato leaves did not differ significantly ($p > 0.05$) between the two pathogens in week ten (Table 4.15).

Table 4.15: Effect of the test pathogens on the leaf number of tomato plants

	Number of leaves				
Pathogen	**Wk2**	**Wk4**	**Wk6**	**Wk8**	**Wk 10**
Alternaria solani	8.70^{a}	10.05^{a}	13.00^{b}	14.20^{b}	16.85^{a}
Phytophthora infestans	6.25^{b}	8.30^{b}	15.30^{a}	16.20^{a}	17.00^{a}
Pr>F	<0.0001	<0.0001	0.000	<0.0001	0.0730^{NS}
SE	0.149	0.181	0.381	0.297	0.306

Means followed by the same letter within a column are not significantly different at p=0.05; Wk - Week; SE - Standard Error; NS – Not Significant

4.4.3 Effects of the Essential Oils on Height of Inoculated Tomato Plant

As expected, the plant height increased with the age of the tomato plants and the treatments influenced the plant height (Table 4.16). For tomato plants inoculated with *A. solani*, there was no significant ($p > 0.05$) variation in plant height between treatments in week two, four and six. In week eight, there was no significant difference between all the essential oils and synthetic fungicide treatments but these treatments recorded significantly ($p < 0.05$) higher plant height than the control. In week ten, tomato plants sprayed with Mexican marigold essential oils recorded the highest plant height (70.10 cm) while the control recorded the lowest (55.77 cm).The plant height of tomato plants sprayed with ginger and garlic essential oil did not vary significantly with those treated with Ridomil® synthetic fungicide (Table 4.16).

Tomato plants inoculated with *P. infestans* also did not differ significantly in height in all treatments during the second week. In week four, the tomato plants in the control treatment recorded significantly ($p < 0.05$) lower plant height than all the other treatments which did not show significant variation among them in plant height. In week six, the plant height

varied significantly ($p < 0.05$) between the treatments with plants in the control treatment being shorter than the plants in other treatments. Tomato plants sprayed with Mexican marigold, ginger and garlic essential oils did not differ significantly in plant height but Mexican marigold treatment was significantly different from both the positive and negative control treatments. Results of week eight and ten showed a similar trend. Mexican marigold essential oil maintained the highest number of leaves in the two weeks. The plant height of tomato plants in the garlic, ginger and positive control (Ridomil® synthetic fungicide) treatments did not have significant difference in their height. The tomato plants in the negative control treatment were the shortest (Table 4.16).

Table 4.16: Effects of essential oils on height of inoculated tomato plants

Test Material	Height of Inoculated Tomato Plants									
	Alternaria solani					*Phytophthora infestans*				
	Wk 2	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**
Control	34.460[a]	46.020[a]	51.348[a]	52.228[b]	55.770[c]	33.153[a]	36.473[b]	40.455[c]	43.810[c]	46.363[c]
Marigold	30.960[a]	46.020[a]	58.323[a]	63.840[a]	70.098[a]	36.408[a]	48.038[a]	63.948[a]	71.733[a]	78.208[a]
Garlic	33.153[a]	47.165[a]	55.908[a]	61.040[a]	64.100[b]	37.653[a]	47.435[a]	56.928[ab]	62.100[b]	68.365[b]
Ginger	34.128[a]	47.375[a]	57.245[a]	60.960[a]	62.640[b]	39.093[a]	49.055[a]	59.463[ab]	64.360[b]	66.953[b]
Ridomil	33.310[a]	47.748[a]	57.485[a]	60.615[a]	61.113[b]	35.980[a]	47.748[a]	53.873[b]	58.955[b]	64.253[b]
Pr>F	0.293[NS]	0.520[NS]	0.099[NS]	0.027	0.003	0.146[NS]	0.004	0.000	<0.0001	<0.0001
SE	1.147	1.208	1.572	1.823	1.585	1.452	1.750	2.101	1.763	1.283
DF	4	4	4	4	4	4	4	4	4	4

Means followed by the same letter within a column are not significantly different at p=0.05; Wk -Week; SE -Standard Error; DF -Degrees of Freedom; NS –Not Significant

4.4.4 Effect of the Test Pathogens on Tomato Plant Height

The plant height increased in all the tomato plants inoculated with the test pathogens (Table 4.17). However, the plant height varied significantly between the tomato plants inoculated with *A. solani* and *P. infestans* in week 2 and week 10. Tomato plants inoculated with *P. infestans* were taller than those inoculated with *A. solani* (Table 4.17). In week 4, 6 and 8, the plant height increased in all the tomato plants but did not differ significantly between the test pathogens (Table 4.17).

Table 4.17: Effect of the test pathogens on plant height

	Height of Inoculated Tomato Plants				
Pathogens	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**
Alternaria solani	33.202[b]	46.865[a]	56.062[a]	59.737[a]	62.744[b]
Phytophthora infestans	36.457[a]	45.750[a]	54.933[a]	60.192[a]	64.651[a]
Pr>F	0.001	0.275[NS]	0.378[NS]	0.700	0.031
SE	0.610	0.710	0.892	0.827	0.651

Means followed by the same letter within a column are not significantly different at p=0.05; Wk - Week; SE - Standard Error; NS – Not Significant

4.4.5 Efficacy of Essential Oils on Control of Tomato Early and Late Blight

Severity of early and late blight diseases caused by *A. solani* and *P. infestans* respectively was significantly reduced by application of essential oils from the selected plants. The essential oils from the three plants gave similar results to the synthetic fungicide used as positive control but varied significantly with the negative control (distilled water). For both pathogens, disease severity in the negative control treatment continued to increase as it decreased in the other treatments (Table 4.18).

Table 4.18: Efficacy of essential oils on early and late blight diseases of tomato

	Disease Severity on Inoculated Tomato plants									
	Early Blight					Late Blight				
Test Material	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**
Control	9.20[a]	18.24[a]	19.66[a]	22.68[a]	24.19[a]	9.87[a]	46.36[a]	66.38[a]	74.80[a]	90.56[a]
Marigold	9.58[a]	9.12[b]	8.55[b]	7.70[b]	6.89[b]	9.75[a]	9.10[b]	7.14[b]	7.00[b]	6.62[b]
Garlic	10.30[a]	9.14[b]	8.55[b]	7.90[b]	7.45[b]	10.14[a]	8.85[b]	7.88[b]	7.30[b]	6.99[b]
Ginger	10.25[a]	8.80[b]	8.31[b]	7.82[b]	7.43[b]	9.82[a]	8.91[b]	8.50[b]	8.38[b]	8.18[b]
Ridomil	9.49[a]	8.69[b]	7.75[b]	6.85[b]	6.44[b]	10.06[a]	8.59[b]	6.72[b]	6.62[b]	6.46[b]
Pr>F	0.118[NS]	<0.0001	<0.0001	<0.0001	<0.001	0.903[NS]	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.276	0.462	0.453	0.332	0.287	0.232	0.754	1.328	0.795	1.080
DF	4	4	4	4	4	4	4	4	4	4

Means followed by the same letter within a column are not significantly different at p=0.05; Wk -Week; SE -Standard Error; DF -Degrees of Freedom; NS – Not Significant

4.4.6 Influence of the Test Pathogens on Disease Severity

The average disease severity on tomato plants inoculated with *A. solani* increased from week two to week four before decreasing in the subsequent weeks. On the other hand, the average disease severity on tomato plants inoculated with *P. infestans* kept increasing throughout the experimental period (Table 4.19). In week two, the disease severity did not vary significantly ($p > 0.05$) between the two test pathogens. From week four to ten, the average disease severity differed significantly between the two pathogens with *P. infestans* recording a higher disease severity than *A. solani* (Table 4.19).

Table 4.19: Influence of the test pathogen on disease severity

	Disease severity				
Pathogens	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**
Alternaria solani	9.77^{a}	10.80^{b}	10.56^{b}	10.59^{b}	10.48^{b}
Phytophthora infestans	9.93^{a}	16.36^{a}	19.32^{a}	20.82^{a}	23.76^{a}
Pr>F	0.308^{NS}	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.109	0.277	0.458	0.279	0.352

Means followed by the same letter within a column are not significantly different at p=0.05; Wk - Week; SE - Standard Error; NS – Not Significant

4.4.7 Efficacy of Essential Oils on Control of Early and Late Blight Diseases

The essential oils from ginger, garlic and Mexican marigold portrayed effective ability to control early and late blight diseases which was not significantly different ($p > 0.05$) from the synthetic fungicide ridomil® (Figures 4.4 and 4.5). However, the efficacy of essential oils and synthetic fungicide was lower on early blight (Figure 4.4.) than on late blight (Figure 4.5) and in both cases, the efficacy increased with increase in the number of applications of the treatments.

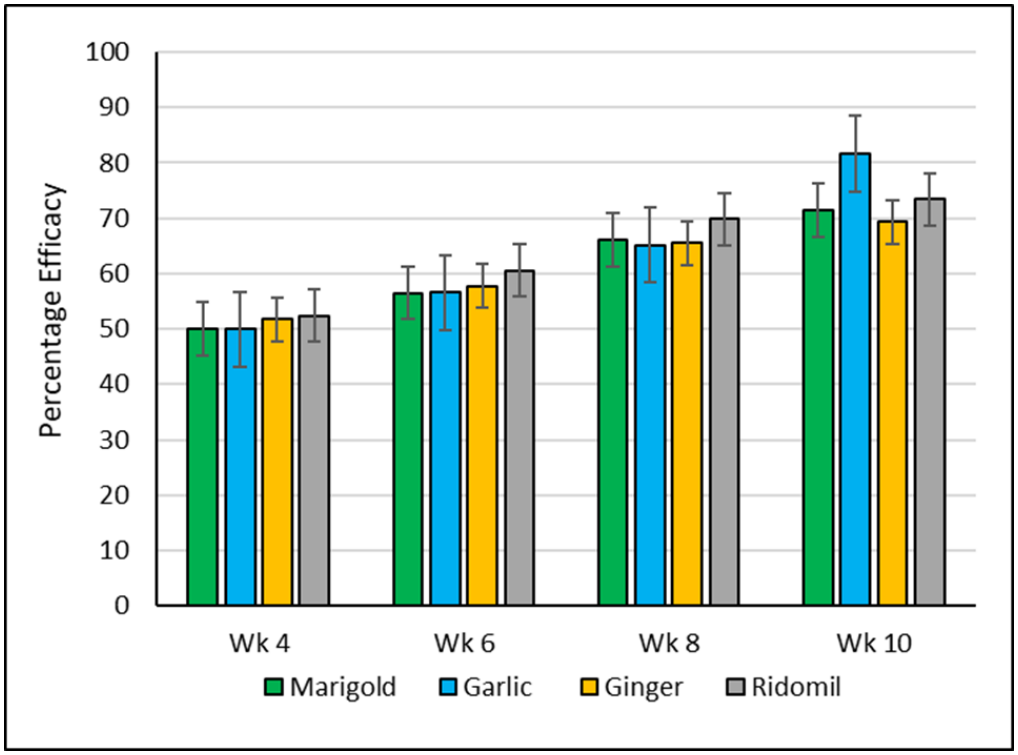

Figure 4.4: Efficacy of essential oils on control of early blight disease. Bars whose error bars are overlapping are not significantly different at p = 0.05. NB: The higher the efficacy, the lower the disease severity.

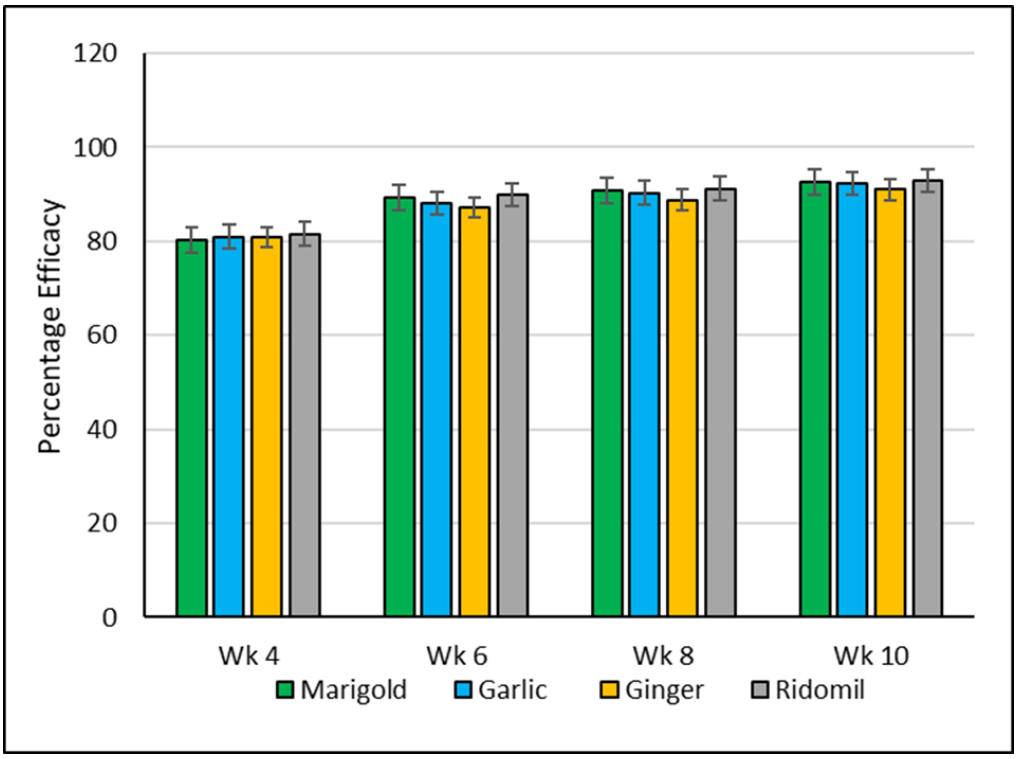

Figure 4.5: Efficacy of essential oils on control of late blight disease. Bars whose error bars are overlapping are not significantly different at p = 0.05. NB: The higher the efficacy, the lower the disease severity.

4.4.8 Effects of Essential Oils on Growth and Yield of Tomato

4.4.8.1 Effects of Essential Oils on Branch Number

The branch numbers obtained across the treatments varied significantly ($p < 0.05$) with tomato plants sprayed with Mexican marigold essential oil recording the highest number of branches among the plants inoculated with *A. solani*. However, the branch number in the Mexican marigold treatment did not differ significantly ($p > 0.05$) from the ones recorded from tomato plants sprayed with ginger (Table 4.20). The rest of the treatments did not differ significantly in tomato branch numbers. Among tomato plants inoculated with *P. infestans,* the number of branches varied significantly ($p < 0.05$) between treatments with the negative control recording the lowest branch number while tomato plants sprayed with Mexican marigold essential oil showed more branching but did not differ significantly with the branching in garlic and ginger treatments. However, tomato plants sprayed with Mexican marigold essential oil had significantly higher number of branches compared to the Ridomil synthetic fungicide (positive control) treatment (Table 4.20).

4.4.8.2 Effects of Essential Oils on Days to Flowering

The treatments significantly ($p < 0.05$) influenced the number of days to flowering on the tomato plants inoculated with *A. solani* (Table 4.20). Plants sprayed with Mexican marigold essential oil took significantly longer time to flower followed by Ridomil®synthetic fungicide treatment and the negative control. Tomato plants sprayed with ginger and garlic essential oils took shorter time to flower as compared to the other treatments. Days to flowering did not differ significantly ($p > 0.05$) among tomato plants inoculated with *P. infestans* (Table 4.20).

4.4.8.3 Effects of Essential Oils on Fruit Number

The number of tomato fruits varied significantly ($p < 0.05$) between treatments and the control (Table 4.20). Among tomato plants inoculated with *A. solani,* the control recorded the lowest number of fruits while the fruit number did not differ significantly ($p > 0.05$) between tomato plants sprayed with garlic, ginger, Mexican marigold essential oils and Ridomil®synthetic fungicide (Table 4.20). Among tomato plants inoculated with *P.*

infestans, all the treatments recorded significantly higher fruit weight than the negative control but only Mexican marigold treatment was significantly different from the positive control (Ridomil®synthetic fungicide) in terms of fruit number (Table 4.20).

4.4.8.4 Effects of Essential Oils on Fruit Weight

The average fruit weight also varied significantly ($p < 0.05$) between the treatments (Table 4.20). For the plants inoculated with *A. solani*, the negative control treatment recorded the lowest fruit weight compared to the other treatments all of which did not differ significantly in fruit weight. For the plants inoculated with *P. infestans*, Mexican marigold treatment recorded the highest fruit weight which was not significantly different from fruit weight recorded in garlic treatment. Tomato plants sprayed with garlic and ginger essential oil did not differ significantly from the ones sprayed with Ridomil® synthetic fungicide. The control recorded significantly lower fruit weight than all the other treatments (Table 4.20).

Table 4.20: Effects of essential oils on growth and yield of tomato

	Alternaria solani				*Phytophthora infestans*			
Test Material	**Branch Number**	**Days to Flowering**	**Fruit Number**	**Fruit Weight**	**Branch Number**	**Days to Flowering**	**Fruit Number**	**Fruit Weight**
Control	3.625^{b}	51.083^{b}	2.938^{b}	31.113^{b}	0.688^{c}	57.938^{a}	1.563^{c}	5.035^{c}
Garlic	3.625^{b}	47.500^{c}	7.438^{a}	41.193^{a}	3.875^{ab}	55.375^{a}	5.250^{b}	42.333^{ab}
Ginger	3.938^{ab}	48.000^{c}	6.500^{a}	44.653^{a}	4.063^{ab}	53.688^{a}	6.438^{b}	36.225^{b}
Marigold	4.438^{a}	55.500^{a}	10.813^{a}	43.918^{a}	5.105^{a}	57.375^{a}	12.125^{a}	46.518^{a}
Ridomil	3.313^{b}	51.750^{b}	8.938^{a}	43.263^{a}	3.000^{b}	53.918^{a}	7.438^{b}	36.765^{b}
Pr>F	0.019	0.001	0.005	0.000	0.001	0.098	<0.0001	<0.0001
SE	0.165	0.896	1.029	1.452	0.470	7.017	0.799	2.018
DF	4	4	4	4	4	4	4	4

Means followed by the same letter within a column are not significantly different at p=0.05; SE - Standard Error; DF - Degrees of Freedom; NS – Not Significant

4.4.9 Effects of the Test Pathogens on Growth and Yield of Tomato

The branch number, days to flowering and fruit number did not differ significantly ($p>0.05$) between tomato plants inoculated with the different test pathogens (Table 4.21). However, the tomato fruit weight differed significantly ($p<0.0001$) between the test pathogens with tomato plants inoculated with *P. infestans* recording a lower fruit weight (Table 4.21).

Table 4.21: Effects of the pathogen on growth and yield parameters of tomato

	Tomato Growth and Yield Parameters			
Pathogens	Branch Number	Days to Flowering	Fruit Number	Fruit Weight
Alternaria solani	3.788^{a}	50.767^{a}	7.325^{a}	40.828^{a}
Phytophthora infestans	3.346^{a}	49.659^{a}	6.563^{a}	33.357^{b}
Pr>F	0.058^{NS}	0.737^{NS}	0.209^{NS}	<0.0001
SE	0.159	2.313	0.420	0.811

Means followed by the same letter within the column are not significantly different at p=0.05; SE - Standard Error; NS – Not Significant

4.5 Correlation of Growth and Yield Parameters in Diseased Tomato Plants

In the plants inoculated with *A. solani*, the plant height had a significant ($p < 0.05$) positive correlation with the number of leaves, fruit number and fruit weight but was negatively correlated to disease severity (Table 4.22). There was no significant ($p > 0.05$) correlation between plant height and branch number and days to flowering. The leaf number was also positively correlated to the fruit number and fruit weight, negatively correlated to disease severity but had no significant ($p > 0.05$) correlation with branch number and days to flowering. Disease severity was also found to negatively influence the fruit number and fruit weight but did not significantly ($p > 0.05$) affect the branch number and days to flowering. The branch number had no significant ($p > 0.05$) effect to days to flowering. Likewise, the days to flowering had no significant ($p > 0.05$) effect to fruit number and fruit weight (Table 4.22). In the plants inoculated with *P. infestans*, all the growth and yield parameters were significantly ($p < 0.05$) and positively correlated to each other and negatively correlated to disease severity (Table 4.22).

Table 4.22: Correlation between growth, yield and disease severity

Variables	Disease Severity	Leaf Number	Plant Height	Branch Number	Days to Flowering	Fruit Number	Fruit Weight
Disease Severity		**-0.610**	**-0.869**	**-0.778**	**-0.657**	**-0.690**	**-0.945**
Leaf Number	**-0.738**		**0.738**	**0.734**	**0.515**	**0.745**	**0.751**
Plant Height	**-0.642**	**0.670**		**0.841**	**0.610**	**0.833**	**0.935**
Branch Number	-0.202	-0.260	-0.293		**0.463**	**0.666**	**0.830**
Days to Flowering	0.013	-0.185	0.251	-0.173		**0.629**	**0.746**
Fruit Number	**-0.680**	**0.453**	**0.559**	0.085	0.241		**0.782**
Fruit Weight	**-0.834**	**0.568**	**0.543**	0.117	-0.042	**0.579**	
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Values in bold are different from 0 with a significance level alpha=0.05. The upper and the lower values represent the effect of *P. infestans* and *A. solani* inoculation respectively

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Morphological and Molecular Characterization of the Pathogens

In the current study, the pathogen isolates that were thought to be twenty-four *A. solani* and sixteen *P. infestans* isolates, until molecular identification revealed that they were mixed with other closely related fungal species. Two main species of *Alternaria* (*A. solani* and *A. alternata)* were obtained from the infected tomato leaf samples collected from tomato farms in Mwea. *A. solani* was more prevalent than *A. alternata* with four of the isolates. Therefore, morphological characterization provided a good lead towards species identification but could not specifically identify the isolates to species level. The cultural, pathogenic, molecular variability and morphological of *A. solani* has been documented by several studies in various countries (Ahmad, 2002; Naik *et al*., 2010). Morphological fungal characteristics such as colony colour, shape, texture of colonies and size of the conidia have been used to differentiate *Alternaria* species (Simmons, 2007). Okayo *et al.* (2020) noted that morphological classification of fungal species lacks precision but it is necessary in aiding the grouping of fungal isolates into groups which allows easier scrutiny using advanced molecular or biochemical characterization.

The study revealed high morphological and genetic diversity among *A. solani* isolates. Several researchers have reported variations in fungal morphological, genetic diversity and pathogenicity (Lourenco *et al*., 2011; Meng *et al*., 2015). Macroscopic features on the growth medium such as colony (front) colour, growth pattern, substrate (reverse) colour and growth margin colour showed variability among the isolates in the current study. Similar results have been documented by Koley *et al*. (2015), Arunakumar (2006), Brook and Dennis (2012), and Hubballi *et al*., (2010). The microscopic features of mycelia and conidia of *A. solani* observed in this study were similar to those reported by Ahmad (2002), Brooke and Dennis (2012) and Naik *et al*. (2010). The high variations observed may be as a result of different strains of *A. solani* that existed in the research area.

This study was successful in identifying *A. solani* and *P. infestans* isolates to species level through amplification and subsequent sequencing of the Internal Transcribed Sequences (ITS) region. The pathogen DNA band sizes generated from the PCR product with ITS 1 and ITS 4 primers were about 580 bp for the two test pathogens. These results were in correlation with those of Loganathan *et al*. (2014) who used ITS 1 and ITS 4 to amplify *A. solani* DNA and observed a band of 580 bp. Manter and Vivanco (2007) and Embong *et al*. (2008) reported that diverse *A. solani* species generated bands ranging from 400-600 bp. Different DNA band sizes have been obtained by different studies using different primers on the same fungal pathogen. For example, with Random Amplified Polymorphic DNA (RAPD), *A. solani* DNA yielded bands ranging between 100-2000bp (Tiwari and Chitora, 2013). Primer set H3-1a/H3-1b used on *A. solani* DNA amplified the bands to 580 bp (Zheng *et al*., 2015). The Genomic DNA of *P. infestans* amplified using TUBUF2 and TUBUR1 primers resulted with bands of about 990bp (Wesam *et al*., 2013). *P. infestans* genomic DNA amplified using ITS 3 and ITS 4 primers yielded band of 612bp (Khalid *et al*., 2018) and with PINF and ITS 5 primers, *P. infestans* DNA yielded bands of 600 pb (Trout *et al*., 1997)

Sequencing and similarity matching of the isolates with available accessions through blasting on the NCBI database enabled identification of *A. solani* clones and *P. infestans* strains that were available in the study area. These findings indicated that different clones of *A. solani* existed in the study area which is not typical of a species that is known to only reproduce asexually. Clone 105 was the most prevalent with a total percent frequency of 52.18% followed by clone 185 (17.39%) and clone 43 (8.68%). The results also revealed the presence of one unidentified clone of *A. solani* in the study area. In addition, the results portrayed close association between *A. solani* and *A. alternata*. The two pathogens (*A. alternata* and *A. solani*), were reportedly isolated from blight infected plants in several previous studies. Zheng *et al*. (2015) reported the presence of *A. solani* and *A. alternata* in China causing blight in potatoes. Other *Alternaria* species reported in China were *A. dumosa, A. infectoria, A. grandis* and *A. interrupta* that also caused foliar diseases but were absent in Mwea, Kenya. According to Hausladen and Leiminger (2007), the same fungal pathogens (*A. alternata* and *A. solani*) were found to cause foliar diseases in Germany. *A.*

alternata was also reported to cause early blight diseases in India with 80 -90% disease incidence on susceptible tomato plants (Loganathan *et al*., 2014). However, in Sweden, *A. alternata* was absent in foliage with early blight symptoms (Blixt and Andersson, 2010).

High genetic diversity of *A. solani* was expressed in isolates from Brazil, Turkey, Cuba, South Africa, United States, Greece, China, Russia and Canada based on vegetative compatibility groups (Van der Waals *et al*., 2004). This correlates with the results of this study where *A. solani* showed a high genetic diversity. Use of molecular markers such as Random amplified microsatellite markers (Van der Waals *et al*., 2004), Random amplified polymorphic DNA markers (Leiminger *et al*., 2013), Amplified fragment length polymorphisms (Lourenco *et al*., 2011), and SSR markers (Meng *et al*., 2015) revealed high genetic variability among the fungal isolates. *A. solani* isolates collected from the same farmers' fields showed genetic diversity (Leiminger *et al*., 2013). According to Craven *et al*. (2008), genotypic variation in *A. solani* is caused by the ability of its mycelia to interconnect by bridges made through hyphal fusion that enable the distribution of water, nutrients and signaling molecules all over the colony.

Genetic diversity is also contributed by mutations, recombinations or movement of the fungal pathogen over long distances (Van der Waals, *et al*., 2004). McDonald and Linde (2002) suggested that the evolution of fungal pathogen populations was probably due to mutations, selection and gene flow and this may have influenced evolution of *A. solani* populations. Movement of tomato seedlings from one farm to another may contribute to variation in the fungal isolates examined (Weir *et al*., 1998). Results of Chaerani and Voorrips (2006) showed genetic variation among fungal isolates collected from different lesions of the same leaflet. Genetic variation of *A. solani* is also caused by heterokaryosis which occurs as a result of hyphal anastomosis (Chaerani and Voorrips, 2006). Nuclear migration through septal pores between cells of mycelia, conidiophores, conidia and cells connecting the structures allows dissociation of unlike nuclei leading to homokaryosis that brings about variation (Chaerani and Voorrips, 2006). These factors may also have contributed to the genetic diversity of *A. solani* in Mwea, Kenya.The ability of *A. solani* to maintain exorbitant genetic diversity allows the pathogen to survive in changing

environmental conditions and this enables the fungi to develop resistance to fungicides thus causing significant losses in tomato and potato. The results of this study did not agree with those of Brooke and Dennis (2012) who reported that there is no genetic variation in *A. solani*.

Out of the sixteen isolates that were suspected to be *P. infestans*, only eleven were *P. infestans*. Three of them belonged to unspecified species of the genus *Phytophthora* while two of them belonged to *Fusarium equiseti*, a soil inhabiting fungus that causes wilt disease in different vegetable plants (Akbar *et al*., 2018). All the eleven isolates that were positively identified as *P. infestans* were found to be Strain A2 indicating high dominance of this strain in the study region. High occurrence of A2 strain of *P. infestans* has been reported in several parts of the world. A study conducted by Li *et al*. (2013) revealed that 91% of the *P. infestans* isolates were A2 mating type. Njoroge *et al*. (2018) also reported dominance of A2 strains in Kenya. Zhao Qing *et al*. (2016), revealed 62% of A2 *P. infestans* isolates.

A study by Han *et al*. (2013) also revealed increased frequency of A2 than A1, which agrees with the results of the current study that had the highest number of A2 strain. The *P. infestans* A2 strains obtained from this study did not have any genetic variation. These results agree with those of Cardenas *et al*. (2011) who reported low genetic diversity among *P. infestans* isolates from different regions of Venezuela and Colombia. Similarly, Wu *et al*. (2012) observed low genetic diversity among 134 strains of *P. infestans* from four provinces in China. The low variability among *P. infestans* isolates has been attributed to clonal populations of the pathogen present in the target regions (Cardenas *et al*., 2011). However, Han *et al*. (2013) reported high genetic diversity among *P. infestans* field isolates in China despite their high frequency of self-fertility.

The possible sources of genetic variation in *P. infestans* include gene conversion, mitotic crossing over and extra chromosomal elements (Abu-El Samen *et al*., 2003). Other researchers explained the following factors that affect genetic diversity of *P. infestans*: the presence of sexual mating types which encourage genetic exchange (Grunwald *et al*.,

2001); the presence of many wild species of the host of the genus *Solanum* (Spooner *et al*., 2004) and ideal climate with favourable temperatures ideal for the pathogen development (Krause *et al*., 1975). Diversity of *P. infestans* may also be contributed by migration and sexual recombination of the pathogen (Cooke *et al*., 2003). Other sources of genetic diversity include; genetic recombination of A1 and A2 mating types, segregation of heterokaryons (Pipe *et al*., 2000), self-sterility (Smart *et al*., 1998), aneuploidy (Carter *et al*., 1999), polyploidy (Tooley and Therrien, 1991) and zoospore-mediated hyphal fusion (Judelson and Yang, 1998). These factors did not have effect on *P. infestans* in Mwea because their genetic variation remained low.

5.1.2 Characterization of Essential Oils

From the results of the current study, it was noted that the major classes of chemical compounds present in essential oils of the test plants with a higher percentage were; terpenes, esters, ketones, organosulfurs and alkanes. Their percentage composition varied in terms of quantity and chemical composition between the test plant species. These results were in agreement with those of Dhifi *et al*. (2016) who reported that essential oils contain diverse chemical classes such as terpenes, aldehydes, alcohols, ketones, ethers, amines, esters, phenols, amides and terpenes were the majority. In all the four essential oils from tickberry garlic, Mexican marigold and ginger, terpenes were the majority. These results also corroborate with those of Swamy *et al*. (2019) and Matos *et al*. (2019) who reported that terpenes constitute about 50-95% of the essential oils but contribute less to flavour and fragrance of the essential oils. Similar to the results of this study, Murugesan *et al*. (2016) also revealed that ginger essential oil had more terpenes.

Essential oil extracted from flowers and leaves of tickberry growing in Cameroon and Madagascar contained curcumene (24.7%), Davanone (15.9%) and β-caryophyllene (13.3%) as the major compounds (Ngassoum *et al*., 1999). Similar compounds that were grouped in the class of terpenes were also identified in the current study but were not the major compounds in tickberry essential oils. In Brazil, tickberry essential oil contained limonene, α phellandrene, germacrene D, and Zingiberene as the major compounds (da Silva *et al*., 1999). However, in the current study, the major compound in tickberry

essential oil was 3-carene and did not have zingiberene. Tickberry essential oil contained Germacrene D, bicyclogermacrene and β-caryophyllene which have been reported as chemical markers of tickberry species (Singh *et al*., 2016). Diversity in the compounds of plant essential oils could be attributed to plants' geographical location (Chalchat *et al*., 1993; Sanli and Karadogan, 2017) nature of chemotypes (Gil *et al*., 2000; Golparvar and Hadipanah, 2016), plant parts harvested, stage of growth of harvested plants (Yuan *et al*., 2016; Murarikova *et al*., 2017), soil nutrient status and soil type (Singh *et al*., 2016), sunlight, climate, methods of harvesting and oil extraction method (Fernandez and Ormeno, 2012). The time of the year that the plant material was harvested in order to extract the oil may also determine the concentration of the essential oil compounds such as phenolics (Malatova *et al*., 2011). This study did not consider the different parts of the tickberry or geographical locations. The oil was extracted from the leaves.

Hydro distilled ginger essential oil from 10 months old ginger rhizome cultivated in North East India contained no zingiberene compounds (Sharma *et al*., 2002). Many variations have been reported in plant essential oils. A report of Agrawal *et al.* (2001), showed that curcumene was the major constituent of ginger oil while Menut *et al.* (1994), reported citral to be major constituent of the ginger essential oil. Others reported zingiberene as the major constituent (Raina *et al*., 2005; Fakim *et al*., 2002). In the current study, the major compound in ginger essential oil was Corymbolone (3.58%) belonging to the family of Ketones, followed by zingiberene (3.54%). Such variations in chemical constituents of essential oil from similar plant parts could be influenced by environmental (climate and soil factors), developmental and genetic factors. Essential oil composition and yield differ significantly with production conditions (Cannon *et al*., 2013), variety, population or cultivars studied (Galambosi and Peura, 1996). This study did not put these factors into consideration.

Organosulfur compounds identified in the current study in garlic essential oil was similar to those identified by other studies (Edris and Fidel 2002; Pyun and Shin, 2006). The organosulfur compounds are antifungal, antibacterial, anti-inflammatory, antioxidant, anti-carcinogenic, antidiabetic, cardioprotective and neuroprotective (Park *et al*., 2012)

therefore important in drug and food industries. Post-harvest practices in garlic influence the chemical composition of garlic essential oils (Ichikawa *et al*., 2006). According to Abu-Lafi *et al*. (2004) garlic oil had more sulfur compounds than terpenes. This observation does not agree with the results of this study that revealed that garlic essential oil had more terpenes than the sulfur compounds. Rao *et al*. (2007) reported that terpenes were not common in garlic essential oil and in most cases only little amounts of monoterpene hydrocarbons like limonene and sabinene were identified. This observation does not agree with the results of the current study because sabinene and limonene were not detected in the garlic essential oil used in this study. Oven drying of garlic before essential oil extraction is associated with loss of terpenes in the essential oil (Rao *et al*., 2007). The current study used fresh garlic and that would be the reason why there were more terpenes because there was no loss of the compounds through drying. Roberta and Guido (2020) identified E-E farnesal in their garlic essential oil but the compound in the current study was only found in ginger essential oil.

Spathulenol, a class of terpenes known for immunomodulatory activities was reported in Mexican marigold essential oil (Martins *et al*., 2010). Caryophyllene was present in all the essential oil of the test plants in the current study. Mexican marigold essential oil obtained from aerial parts contained major compounds such as Limonene, (Z)-$_{\beta}$-ocimene, (E)-$_{\beta}$-ocimene, (Z)- and (E)-tagetones, dihydrotagetone, (Z)- and (E)-tagetonones (Marotti *etal*., 2004; Singh *et al*., 2016). In the results of this study (E)- and (Z)-targetones, dihydrotagetone, (E)- and (Z)- tagetonones were identified but were not the major compounds in Mexican marigold essential oil. The major compound identified in the Mexican marigold, essential oil in the current study were: Chrysantenyl 2-methuylbutanoate (3.06%) which was in the category of esters,3,5Papaveroline, 1,2,3,4-tetrahydro-3-O-methyl- (3.02%), which was an alkaloid and Dimethylanisole (3.01%) in the class of aromatics.

The commonly studied compounds are phenolics (Malatova *et al*., 2011), terpenes (Murugesan *et al*., 2016), organosulfurs (Rao *et al*., 2007), while compounds such allethrin, antihistamine, silane among others have scanty information. Comparing the compounds in

the essential oils of the four test plants in the current study with those that have been studied in other parts of the world, indicated that the chemical composition of the essential oils varied greatly.

5.1.3 *In-vitro* Anti-Microbial Activity of the Plant Crude Extracts and Essential Oils

From the current study, it was evident that all the plant crude extracts tested in the study (garlic, ginger, Mexican marigold and tickberry) were successful in reducing the growth of *A. solani* and *P. Infestans* compared to the control. However, the inhibition of the pathogen growth varied with the type of the test plant extracts, with garlic showing the highest rate of growth inhibition on the two test pathogens. The results attained confirmed that garlic, ginger, tickberry and Mexican marigold crude extracts contain antimicrobial compounds by their ability to reduce mycelial growth of the test pathogens. The results of the current study are in concurrence with those of other authors such as Daniel *et al*. (2015), who reported that garlic extract effectively inhibited *Botrytis cinerea in-vitro*. Extracts from ginger were found to have fungicidal properties and worked against *Fusarium* spp, *Curvularia* spp, and *Colletorichum* spp (Krishnapillai, 2007). Hot water extracted extracts from ginger were found to inhibit the extension of *Aspergillus niger*, *A. flavus* and *F. oxysporum* in culture and decreased decaying of yam tubers (Okigbo and Nmeka, 2005). Tick berry extracts were reported to effectively inhibit the growth of *P. infestans* (Amienya and Onunze, 2015) and *Colletotrichum falcutum* that causes red rot disease of sugarcane (Sreeramulu *et al*., 2017).

Leaf extracts of tick berry inhibited fungal spore germination of *Riccia billardieri* (Chaudhary *et al*., 2007). Mexican marigold extracts were reported to significantly suppress root-knot nematodes in tomato (Aminu-Taiwo and Fawole, 2018) and also have antibiotic impact against *Staphylococcus aureus* and *Escherichia coli* (Uzabakiriho *et al*., 2015). Extracts from ginger were found to have fungicidal properties against *Fusarium oxysporum* f. sp. *lycopersici* (Rawal and Adhikari, 2016). Extracts from ginger were also reported to have fungicidal properties and worked against *Fusarium* spp, *Curvularia* spp, and *Colletorichum* spp (Krishnapillai, 2007). Rodino *et al*. (2014), performed an experiment using Mexican marigold crude extracts and reported reduction of mycelia

growth of *Rhizoctonia solani*. In Nigeria, Mexican marigold extracts significantly suppressed soil nematode population and galls on cowpea roots and was suggested as a mitigation measure against nematode pests in cowpea (Olabiyi and Oyedunmade, 2007).

Different concentrations of plant crude extracts have been used by different studies in efficacy studies. In the current study, 5 ml of each plant extracts were used to amend 500 ml each of PDA and V8 agar. Sifat and Monjil (2017), reported that 10 % concentration of crude garlic extract caused 95.5% growth inhibition of *Rhizoctonia* spp. while 20% concentration portrayed excellent reduction in mycelial growth of *Rhizoctonia solani* that causes sheath blight in rice. Bhuiyan *et al*. (2008), found 20% concentration of garlic extracts being effective against growth of *Colletotrichum dematium*. Slusarenko *et al.* (2008), reported that allicin from garlic effectively controlled *Alternaria* spp. in carrot and *Phytophthora* leaf blight of potato. Garlic and ginger extracts inhibited growth of *Penicillium* spp. and *Aspergillus* spp in *in-vitro* (Agi and Azike, 2019). Three (3) gm/20ml concentration of ginger crude extract reduced post-harvest fungal rots in tomato fruits (Chuku *et al*., 2010).

From the current study, it was evident that the two test pathogens responded differently to different test plants crude extracts. *Alternaria solani* was more susceptible to ginger extracts than *Phytopthrora infestans* while *P. infestans* was more susceptible to garlic crude extracts than *A. solani*. According to the results of the current study, ginger extracts or its essential oil would be the most suitable for control of early blight. Garlic extracts or its essential oil would be the most suitable for control of late blight. This observation indicates that the antimicrobial efficacy of the crude extracts may be determined by the sensitivity of the target pathogens to the extracts. The active antimicrobial compounds have been reported in previous studies. Vidyasagar and Tabassum (2013), reported that Mexican marigold plant species contained compounds such as piperitone and piperitonene which inhibited fungal mycelial growth by modifying the structure of the mycelia. Sarfraz *et al*. (2020), reported that allicin from garlic effectively controlled *Alternaria brasicicola, Verticillium dahlia, Verticillium longisporum* and *B. cinerea*. Reports of Mahady *et al*. (2003), showed that ginger has shogaols that contain both antifungal and antibacterial

properties. Tickberry reportedly contain flavonoids, alkaloids and tannins that inhibit the growth of *Alternaria alternata* (Singh and Sristava, 2012; Mariajancyrani *et al*., 2014). These compounds were also identified in the essential oils used in this study.

The activity of crude extracts varies depending on the test plant material, the solvent used and the method of extraction (Bandor *et al*., 2013; Brusotti *et al*., 2013; Mahlo *et al*., 2013). Results of the current study showed that extraction solvent had a significant influence on the effectiveness of the crude extracts. In the current study, the most suitable extraction solvent in improving the performance of the test plant extracts against the test pathogens was ethanol followed by methanol and then water. Similar results were reported by Singh and Srivastava (2012) with tickberry where ethanol extracted extracts were the most effective in reducing the growth of *Alternaria alternata* followed by methanol extracted extracts and then water extracted extracts. The findings of the current study were in concurrence with those of Wongkaew and Sinsiri (2014), who documented that when ethanol is used as a solvent for extraction of plant extracts, a higher level of effectiveness is realized as compared to other solvents.

In-vitro anti-bacterial activity of ethanol extracted extracts of tickberry were reported to restrain the growth of both gram positive bacteria and gram negative (Xavier and Arun 2007). The high level of effectiveness in ethanol as a solvent is attributed to the fact that ethanol is a polar solvent and produces a higher concentration of phenolics and the quality of the extracts is better when dilution is done with water (Bandor *et al*., 2013). The low efficacy of aqueous plant extracts is attributed to the fact that water does not extract non polar active compounds in plant materials (Masoko *et al*., 2005). The use of water as an extraction solvent also leads to addition of impurities that lower the quality of the extracts (Bandor *et al*., 2013). However, the results of the current study differed with those of Dabur *et al*. (2007) who reported that tickberry water extracts had a higher efficacy than organic solvents extracts.

From the results of the current study, essential oils from all the four test plant extracts were effective in restraining the growth of the test pathogens significantly in comparison to both

positive and negative controls. The efficacy of different essential oils from different plant species has been reported. Saikia and Sahoo (2011) reported that essential oils of tickberry had prominent bacterial activity against all bacteria strains tested and gram positive bacteria were the most sensitive strains. Essential oils of tickberry were reported to exhibit significant antibacterial activities against different strains of bacteria (Machado *et al*., 2012; Sousa *et al*., 2012). According to Dua *et al*. (2010), essential oil from tickberry leaves have insecticidal activity against mosquitoes. Essential oils of tickberry exhibit insecticidal, anti-feedants, anti-microbial and anti-helmintic properties (Singh *et al*., 2012). Insecticidal properties of tick berry essential oil have been reported to be effective against *Sitophilus* spp (Zoubiri and Baaliouamer, 2011; Mohamed and Abdelgaleil, 2008). Although the insecticidal properties were not tested in the current study, it shows that the essential oils from the test plants have broad-spectrum effects.

According to Koul *et al*. (2008), garlic oil was exceedingly lethal to the eggs of diamond back moth. Garlic essential oil was effective against free-living soil inhabiting nematodes (Block 2010). The oil also had insecticidal activity against mealworm beetle in laboratory conditions where it caused mortality and repellence of adult, pupa and larval stages of the mealworm beetle (Isman, 2006). Mahmoud *et al*. (2013), reported extensive inhibitory action of garlic essential oil on *F. solani*, *M. phaseolina*, *R. solani* and *S. rolfsii*. Essential oils of ginger were found to have a substantial fungicidal impact on the fungi *Penicillium sp*., *B. cinerea, A. niger* and *R. nigricans* (Stoyanova *et al*., 2006), *Penicillium expansum*, *A. flavus*, *A. alternata* and *F. oxysporum* (Sharma *et al*., 2013). This was similar to the results from this study.

Ginger essential oil was also reported to hinder the growth of *Botrytis cinerea* of grapes during storage with efficacy increasing with increase in concentration (Tripathi *et al*., 2008). Ginger oil was reported to inhibit the mycelia growth of *A. parasiticus* and *A. flavus* (Silva *et al*., 2012). Ginger essential oil was also found to be effective in hindering the growth of *F. oxysporum*, *R. solani* and *B. theobromae* causing decline disease of guavas (Hamad, *et al*., 2015). According to Saha *et al*. (2012), essential oils of Mexican marigold have antifungal properties that worked against *Pyricularia grisea*, *Sclerotium rofsi*, *A.*

solani and *F. oxysporum*. The efficacy of essential oils is connected to synergistic interactions between components, functional groups and composition (Dorman and Deans, 2000). The different amount and types of anti-oxidative compounds present in essential oils are thought to bring the differences in anti-oxidative activity of different essential oils (Burt 2004; Kordali *et al*., 2005).

The crude extracts and essential oils of all test plants in the current research were useful in inhibiting the growth of the test pathogens. However, essential oils had a higher antimicrobial activity as compared to the crude extracts of the same plants. Reports of Kimbaris *et al*. (2009) indicated that garlic extracts and essential oils have both ovicidal and larvicidal properties against a wide range of insects including mosquitoes. According to the results obtained in the current study, it was noted that the efficacy of the test plant to the test pathogen was dependent on the method of extraction and the type of the plants used. Essential oils were more effective, followed by ethanol extracted extracts. These results agree with those of Vidyasagar and Tabassum (2013) and Wongkaew and Sinsiri (2014) who reported that the method of extraction, concentration of the extracts, origin of plants, sensitivity of the strains of the test pathogens, the solvent used in extraction and the active compounds present in the test plants determine the effectiveness of the plant extracts against the test pathogens. The results of this study also indicated that there was significant interaction between the test pathogen and the extraction method in all the test plants

5.1.4 *In-vivo* Efficacy of Essential Oils against Early and Late Blight of Tomato

In the current study, essential oils were found to influence the number of leaves in the tomato plants as compared to the control. Tomato plants sprayed with Mexican marigold essential oil exhibited a higher number of leaves. The aroma from the oils was noted to repel whiteflies. Mexican marigold repelled whiteflies more than ginger and garlic and this could have contributed to the tomato plants sprayed with Mexican marigold having more number of leaves. Increase in the number of leaves could be attributed to the chemical composition of the essential oils improving the growth of the plant. According to Mahmoud *et al*., (2013) garlic essential oil had a significant effect in increasing the survival rates of peanut plants infected by damping off disease. On the other hand, diallyl disulfide

compound from garlic essential oil inhibited growth of leaves in tobacco plants but the roots elongated significantly and the plants showed signs of wilting (Wang *et al*., 2019). In the current study, the treatments (ginger, garlic, Mexican marigold essential oils and Ridomil synthetic fungicide) influenced plant height as compared to the untreated control. These findings are in corroboration with those of Ahmad *et al*. (2017) who reported that plants sprayed with extracts of garlic and ginger were taller than the untreated control.

Several essential oils have shown remarkable biocontrol activity against pathogens causing plant diseases. Three essential oils tested in the current study had similar potential with Ridomil®synthetic fungicide in their antifungal properties against early blight and late blight. This is in concurrence with the findings of Lengai *et al*. (2017) and Nashwa and Abo-Elyousr (2012) who reported that ginger, garlic and turmeric extracts had a similar effect to Ridomil® synthetic fungicide in their fungicidal properties against early blight disease in tomato. However, reports of Ngadze (2014) showed that Mexican marigold extracts had a higher antimicrobial effects on late blight disease than garlic. There was no late blight disease on plants sprayed with Mexican marigold extracts, while plants sprayed with garlic extract showed low late blight disease incidence (Ngadze, 2014). Some bio-pesticides induce disease resistance to the systems of the plant which lead to vigorous growth of the plants and thus better production (Naing *et al*., 2013). According to Nashwa (2011), tomato plants inoculated with *A. solani* and sprayed with garlic crude extract recorded 20.8% disease severity which was higher than what was recorded from the current study (10.3%). This difference may have been brought by the fact that essential oils have more active antimicrobial compounds that the crude extracts of the same plant.

Essential oils contain secondary metabolites such as terpenes, organosulphurs, phenols and alkaloids which are antimicrobial and nematicidal (Din *et al*., 2016). Mechanism of disease suppression may be either due to active antimicrobial compounds acting on the pathogen directly by destroying their membranes or the compounds inducing systemic resistance in host plants thus lowering disease development (Kagale *et al*., 2004; Nashwa, 2011; Jambhulkar *et al*., 2016).The antibacterial and antifungal effects of these compounds are as a result of synergistic action of the many compounds and pathogens have negligible

chance of developing resistant races after application of essential oils (El Rasheed and El Rasheed, 2017).

Mexican marigold essential oil contains insecticidal properties and has been used to control *Hyblaea puera* pests causing damage on forest trees (Murugesan *et al*., 2016). According to Gakuubi *et al*. (2016), essential oil from Mexican marigold portrayed antibacterial properties against *Bacillus subtilis* and *Staphylococcus aureus.* The antibacterial properties were influenced by the presence of dihydrotagetone and α-ocimene compounds found in Mexican marigold essential oils which are also toxic to juveniles and eggs of *Meloidogyne incognita*. Mexican marigold essential oil has also been reported to have acaricidal properties by showing 90% efficacy against four species of ticks (Gakuubi *et al*., 2016).

Limonene, α-pinene and 1, 8-cineole found in Mexican marigold essential oil have been reported to have fungicidal properties (Gakuubi *et al*., 2016). This shows that essential oils have broad antifungal and antibacterial inhibitory spectrum. However, the antimicrobial activity of the essential oils may be influenced by the chemical composition, method of extraction and the conditions to which the plant material was subjected to in the preparation for essential oil extraction (Silvia *et al*., 2019). For example, essential oil extracted from fresh ginger comprised of higher concentrations of oxygenated compounds such as neral, 1,8-cineole, α-terpineol, borneol and geranial than essential oils obtained from dried ginger thus low antifungal and antibacterial properties (Silvia *et al*., 2019). The essential oils used in the current study had been extracted from fresh material and contained the oxygenated compounds (Mugao *et al*., 2020b) and this could have been the reason for their higher antifungal activity.

According to Singh *et al*. (2007), phenolic compounds such as shogaols, zingerone and gingerols are responsible for the antimicrobial potency in ginger essential oils. Silvia *et al*. (2017) reported that fungi have a higher sensitivity to ginger compounds than bacteria. Ginger and garlic essential oils showed insecticidal activity by reducing hatchability of cotton leaf worm eggs, prolonging their pupal and larval period significantly, but ginger essential oil was reported to be more effective than garlic oil (Hamada *et al*., 2018).

Furthermore, garlic essential oil insecticidal activity was also exhibited in mealworm beetles where substantial mortality and repellence was observed in larva, pupa and adult stages (Rueda *et al*., 2017). Diallyl disulfide in garlic essential oil has portrayed an antifungal and nematicidal activity against *Phytophthora nicotianae* that cause tobacco black shank disease on tobacco leaves and tomato root knot nematodes (Wang *et al*., 2019). Reports of Mahmoud *et al*. (2013) showed that garlic essential oil portrayed high antimicrobial activity against damping off disease in peanuts. In addition, garlic essential significantly lowered cholesterol biosynthesis by inhibiting HMG-CoA reductase and 14-alpha-demethylase in human beings (Testfaye and Mengesha, 2015).

The controls in the current study showed a higher disease severity of early and late blight diseases than tomato plants sprayed with Ridomil®synthetic fungicide and essential oils. *P. infestans* caused 90% of early blight disease severity on the tomato plants in the control. These results are similar to those reported by Quintanilla *et al*. (2002) who reported that *P. infestans* caused damage above 65% in potato varieties. Disease severity may be influenced by the susceptibility of the cultivars used and the aggressiveness of the test pathogens. *P. infestans* was more sensitive to essential oils and Ridomil® synthetic fungicide as confirmed by Lengai *et al*. (2017). This shows that pathogen species respond differently to the different essential oils.

In the current study, higher fruit yield was obtained in tomato plants sprayed with essential oils and Ridomil® synthetic fungicide as compared to the control. These findings were in contrary with those of Stangarlin *et al*. (2011) who did not observe any significant fruit yield variations in tomato plants sprayed with different plant extracts. However, reports of Nashwa and Abo-Elyousr (2012), showed that different plant extracts improved tomato yields as compared to the control. According to Nashwa (2011), the efficacy of Ridomil® synthetic fungicide and the plant extracts were reflected in the tomato fruit yield when compared with the untreated control. Similar observation was made by Ahmad *et al*. (2017) where maximum yield was achieved from tomato plants sprayed with 20% garlic crude extract. Some plants extracts and essential oils have growth promotion effects which helps in improving the yield of the plants (Naing *et al*., 2013; Culver *et al*., 2012). Rahman *et al*.

(2014) also reported growth promotion effect using microbial pesticides by managing diseases and pests. Increase in tomato fruit yield may also be as a result of reduced diseases and pests during growth and fruit development because the essential oils acted as repellents (Rizvi and Jaffar, 2015). Garlic contains allicin, a compound known to boost plant growth and fruit yield in tomato plants (Ahmad *et al*., 2017).

5.2 CONCLUSIONS

5.2.1 Morphological and Molecular Characterization of the Pathogens

This study successfully isolated and analysed the diversity of *A. solani* and *P. infestans* in tomato growing fields in Mwea, Kirinyaga County, Kenya and concluded that there is higher genetic variability within *A. solani* than *P. infestans* in the area. The study revealed presence of at least four clones of *A. solani* in the area including clone 105 which was the most dominant, clone 185 and clone 43. However, only one strain of *P. infestans* (Strain A2) was identified in the study region. The study also revealed close association between *A. solani* and *A. alternata* as well as between *P. infestans* and other unidentified Phytophthora species. These findings will be useful in development of sustainable strategies to manage the early and late blight diseases in tomato growing areas in Mwea, Kenya.

5.2.2 Characterization of Essential Oil Compounds

The essential oils from tick berry, garlic, Mexican marigold and ginger showed variations in chemical composition. A few chemical compounds such as 3-carene, Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-, Citral, Bicyclogermacrene and Caryophyllene were present in essential oils in all the test plants. Some of the identified compounds have proved to be antimicrobial and therefore qualifying garlic, ginger, tick berry and Mexican marigold essential oils to be used as pesticides for plant diseases and can therefore be exploited for synergistic utility.

5.2.3 *In-vitro* Efficacy of Crude Extracts and Essential Oils

From this study, it was evident that garlic, ginger, tickberry and Mexican marigold essential oils and crude extracts had antimicrobial properties against *Alternaria solani* and

Phytophthora infestans in-vitro. Ethanol was the most suitable solvent for extraction. *Alternaria solani* was more susceptible to ginger crude extracts while *Phytophthora infestans* was more susceptible to garlic crude extracts. Simple methods of essential oil extraction that are less expensive can be used to make the oils available for use as pesticides. Compounds in these test plants, if tapped using the right methods and right solvents can be formulated and made available to farmers to be used as fungicides.

5.2.4 *In-vivo* Efficacy of Essential Oils against Early and Late Blight of Tomato

The study showed that the essential oils from ginger, garlic and Mexican marigold improved tomato vegetative growth, fruit number and fruit yields and effectively reduced early and late blight diseases in tomato. These essential oils can therefore be incorporated in disease management programs thus reducing heavy application of synthetic pesticides which have harmful residues that remain in the produce and also pollutes the environment. This will reduce chemical residue levels in fruits and vegetables thus helping to meet the fruit and vegetable quality requirements by reducing the risks and hazards of toxic fungicides. This will improve access to the best markets of the produce resulting to increased income for the producers and the country at large.

5.3 RECOMMENDATIONS

5.3.1 Recommendations from this Work

1. Tomato producers needs to use a combination of different chemicals in order to manage the variability in the pathogens.
2. Crude extracts and essential oils need to be integrated in disease management because of the many chemical compounds present in them.
3. Garlic should be exploited for the control of early and late blight diseases in tomato

5.3.2 Recommendations for Further Research

1. Further analysis of genetic diversity of *A. solani* and *P. infestans* can be done using other primers and other gene regions apart from ITS regions.

2. Further studies are recommended to investigate the nature of association between *A. solani* and *A. alternata* and also between *P. infestans* and unidentified *Phytophthora* species identified from the current study.
3. Owing to low availability and utilization of bio-pesticides, more research is needed to increase the level and lower the cost of production so as to make them available to farmers at affordable prices.
4. Identification and Isolation of individual compounds that have various antimicrobial effects from the essential oils is necessary
5. More research is needed on the stability and shelf life of the compounds present in the essential oils and the information made available to crop producers.

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